

Cambridge : its manpower and money

THE University of Cambridge, in an annual gesture calculated to bring some relief to an editor beleaguered in the August silly season when government and universities vanish, has just released a small mountain of statistics. Two issues of the *Reporter*, issued on August 7, deal with student numbers and with sponsorship of research within the university. At ten pence, the pair provide a remarkably detailed profile of a university which has often claimed, with some justification, to be particularly distinguished in the sciences.

For several years Cambridge's undergraduate numbers have remained relatively constant. There is at the moment still a swing towards admitting more women but total numbers of students rose only 0.5% last year. On the other hand the numbers of those applying to enter is falling rather rapidly as Table 1 (compiled with the help of a *Reporter* of last year) shows. At present roughly half of those who apply get accepted, not long ago it was barely a third.

Table 1 Trends (percentages) in student numbers from 1973 entry to 1974 entry.

	Applications	Acceptances
English	-18	+3
History	-7	+3
Modern languages	-18	+2
Law	+5	+4
Mathematics	-9	+2
Natural sciences	-16	-10
Engineering	-8	+2
Medical sciences	-3	-1

The swing away from science is manifested in an interesting way. If we can assume certain standards of rationality in the selection procedure we must conclude that the swing is more in quality of student than in quantity of applications. All of this is well known on a national and international scale, and those in provincial universities can be excused a wry smile that Cambridge is beginning to feel the breeze that is a gale in their own laboratories. Once the trend has started downwards, though, the question must be whether the resources of Cambridge are capable of reversing it. The news can only be good for teachers of science in schools, getting increasingly used to being courted by other universities and now, at long last, to be wined and dined at High Table for the favours of their shrinking brood.

The other report puts together expenditures, by sources outside the university, of research projects for the financial year 1972-73. At the detailed level it is a delight. The Department of the Environment spent £150 with Applied Mathematics of "Effect of wind on people". The

Veterinary School received £522 worth of equipment and materials from "Broilers—various". The Science Research Council cheerfully gave £88,651 for "Observational and theoretical astronomy" but needed the justification of "Spectroscopic studies of some metal deficient and some strong line *g* and *k* type stars" before forking out another £40. And what does one make of the Ministry of Defence's total expenditure—£114,000 by our addition, £14,000 by the *Reporter's*? Misprint or deceit?

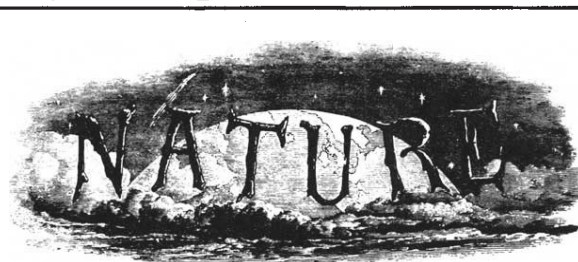
Table 2 Sources of research funding 1972-73.

	£
Government bodies	2,125,000
Charities, trusts, foundations	480,000
Overseas bodies	98,000
Four industries*	81,000
The rest of British industry	60,000
<i>Studentships and capital expenditure excluded</i>	

* Mullard, Beecham, Rolls-Royce, Tobacco Research Council.

Table 2 brings together the sources—our interpretation and our addition. It is worrying, to say the least, that the university has such a restricted financial relationship with industry. Here, surely is a pointer to the mutual suspicion between industry and academics which is such a disagreeable feature of British scientific life. And here, also, is a good place from which an improvement in relations could grow. It would be quite wrong to point a blaming finger specifically at either side, but perhaps when the schoolteachers have had their meal at High Table there might be something left over for industrialists. If not, Cambridge's days as an outstanding university could be numbered.

100 years ago



M. MAREY has recently published the results of experiments undertaken to determine by the graphic method what is the true movement of the legs in walking. His results prove convincingly that the brothers Weber were wrong in assuming that the oscillation of the leg which is not in contact with the ground is the same as that of a pendulum; for when it is represented on a uniformly moving plane, the line drawn is a straight and not a curved one. The movement of the suspended foot is therefore uniform, depending on muscular action, in combination with that of gravity.

From *Nature*, 10, 306, August 20, 1874.



The legacy of the Nixon years

A few hours before he announced his resignation as President of the United States, Richard Nixon vetoed the appropriations bill for the Environmental Protection Agency because he considered it to be inflationary. It was a symbolic last act for an Administration which spent a good deal of time doing battle with Congress over spending priorities. Colin Norman discusses how, for that small section of the population known as the scientific community, which has often been caught in the middle of the budgetary battle, Nixon's last veto was a reminder of what has passed and probably a taste of things to come.

IN his five and a half years in the White House, Nixon did not exactly win the undying support of scientists for his handling of scientific affairs. In fact, throughout his Presidency, cries of alarm from parts of the scientific community have been clearly audible above the background grumblings from most of academe. Among other things, the departed president and his lieutenants have been accused of plunging science into a financial crisis, of relegating some scientific disciplines to second place in the world pecking order, of failing to foresee situations in which science and technology could have been marshalled to help out (such as the energy crisis) and even of ignoring science completely.

The record, however, is a good deal better than many of the criticisms allow, and a good case can be made for the argument that some of the disquiet in the scientific community has its origins in events which took place well before Nixon set foot in the oval office. And it can equally well be argued that many of the science policies and institutional arrangements laid down by President Nixon will survive long after his departure.

Underlying most of the criticisms of Nixon's stewardship of the scientific enterprise is the fact that federal expenditures on science and technology during the past five years have increased at a rate barely sufficient to keep pace with inflation, and in some cases funding has even declined. On top of that, the Administration has gone for some of the scientific community's most cherished programmes, of which biomedical training was perhaps the most prominent. And the final straw

came early last year when Nixon announced that he no longer needed a full-time science adviser in the White House, so he abolished that post along with the Office of Science and Technology and assigned some of its duties to the Director of the National Science Foundation.

Thus, the Nixon Administration's policies were clearly not designed to bring joy to the country's science and engineering laboratories. Nevertheless, scientists and technologists have fared rather better than their colleagues in many other disciplines, and the federal budget for science and technology, which stands at nearly \$19,000 million (\$4,600 million more than it was five years ago) is immense by any measure.

When the Nixon Administration came to power, the unquestioned growth of the science budget which took place during the Eisenhower and Kennedy Administrations had already come to a virtual halt. Thus, the first couple of years of so of Nixon's tenure were marked by painful adjustment from a period of burgeoning growth in science budgets to a period of almost static funding. One consequence was that the job market for scientists rapidly became very tight just when record numbers of scientists were emerging from the academic pipeline, a situation which did little to enhance the new Administration's standing in the eyes of the academic community.

The Administration's response was to cut back on funding for programmes designed to increase the supply of scientists and technologists. In 1971, for example, the proposal was made to eliminate the National Science Foundation's institutional support programme

and to phase out its graduate training programmes. Since those two items were highly cherished sources of funds for universities, which were then facing huge financial deficits, the Administration's academic support slipped a few more notches.

But the budget announced in January 1971 represented, for the first time since 1968, a real increase in federal support for research and development. This was carried through with another boost for science and technology in the budget announced a year later. But, in January 1973, just after his re-election, Nixon slammed the brakes on public expenditure and withheld considerable sums of money promised in the pre-election spending spree, with the result that funding for science again took a hammering. Nixon's support in the scientific community hit another low.

Finally, the budget announced just six months ago promised another round of increases in research and development expenditure, chiefly for the development of energy resources and for the Department of Defense.

The overall pattern of expenditure has therefore been one of stagnation, growth, cutbacks and growth—a situation which has not been conducive to university planning, and which has been unsettling for those who are forced to depend on the government for research grants. Whether or not the fabric of science in the United States has been damaged in the process, is, however, open to question.

The upshot of this slow and sporadic growth in the science budget has been to force a number of painful priority decisions in the basic sciences, as, for example, in space research where a number of promising satellite missions have been dropped, the High Energy Astronomy Observatory has been considerably scaled down in cost and performance and other missions have been deferred to accommodate development of the shuttle in a tight overall budget.

Similarly, particle accelerators have been shut down in order to accommodate the rapid growth in the budget of the National Accelerator Laboratory, and many research grant applications which have been designated as scientifically worthwhile by peer review groups at the National Institutes of Health have gone unfunded for lack of money. In the days of burgeoning growth, such painful choices probably not have been needed.

It has been in the biomedical science community, however, that the cries of anguish over the Administration's science policies have been loudest, and once again, the problems have been

Ford: inherits unhappy science community



White House science adviser
Guyford Stever (left)
and Edward E. David, jun. (right).



exacerbated by a painful transition from periods of rapid growth to stagnation.

Superimposed on the situation, however, is the fact that in the past three years two highly publicised crusades—against cancer and against heart disease—have been launched and enthusiastically supported by both Congress and the Administration. The result has been that the budgets of the National Cancer Institute and the National Heart and Lung Institute have been allowed to grow relatively rapidly, while other NIH institutes have been held back so that many are now receiving less money than they got in 1968. Moreover, biomedical scientists both at NIH and in the Universities have complained bitterly that the Administration has been trying to run the cancer and heart programmes like NASA-style operations to land men on the moon by highly targeted research programmes which have drawn money away from basic research.

On top of all those complaints, the biomedical community has been upset by repeated vetoes by President Nixon of the appropriations bills for the Department of Health, Education and Welfare, which have held up funds for months, and which have kept funding at the levels proposed by the Administration rather than at the more generous levels approved by Congress.

These funding decisions and policy changes have not, however, been taken entirely in a vacuum, for Mr Nixon has also made substantial changes in the science policy machinery in the top echelons of the federal government. In fact, it is those changes which have done most to upset the elder statesmen of the scientific community.

When he arrived in the White House, Nixon inherited an extensive science policy apparatus whose origins dated back to the second World War, but when he departed last week, virtually none of it was left intact.

On the President's immediate staff was a science adviser, and a small policy office called the Office of Science and Technology which provided him with staff support. Lines to the scientific community were kept open by means of the President's Science Advisory Committee, chaired by the science adviser, and filled with a raft of luminaries from the universities. Nixon appointed Lee A. DuBridge, President of MIT as his first science adviser.

DuBridge and the Office of Science and Technology never became a powerful force in the Nixon White House, however, partly because President's

Science Advisory Committee took a number of stands on military matters which were in direct opposition to the Administration's policies, and partly because the rest of the White House just wasn't interested. And, as the influence of the science apparatus waned, the power of the New Office of Management and Budget (OMB) increased, so that it is now the focal point through which White House policy is conveyed to the departments and agencies. It wields tremendous power through the budgetary process.

DuBridge was eventually succeeded by Dr Edward E. David, jun., an engineer from Bell Labs, who departed in January last year after trying to interest the rest of the White House in science, with little success. Within two weeks of David's departure, Nixon scrapped the Office of Science and Technology, the post of Science Advisor to the President and the President's Science Advisory Committee, and designated the Director of the National Science Foundation, Dr H. Guyford Stever, as science adviser to the White House.

Stever has since established in NSF a science policy office and an energy policy office, and he has been given a budget three times larger than that of the defunct Office of Science and Technology. He has also established lines to OMB, and is generally credited with doing a commendable job, given the limits to his power.

The changes irked many scientists, however, who felt that science had been downgraded in national affairs, and many people have also criticised the fact that Stever has specifically been given no mandate to advise on military technology. In response to such complaints, Dr Philip Handler, President of the National Academy of Sciences, established recently a special

blue ribbon panel under the chairmanship of Dr James Killian, Eisenhower's first science adviser, to look into the workings of the present science policy apparatus.

Not surprisingly, the Killian panel recommended that a science advisory council should be re-established in the White House, and that the present arrangement is unsatisfactory since Dr Stever, being head of a small science agency, is not in a strong enough position to orchestrate the vast federal science bureaucracy. Since there was absolutely no chance that Nixon would reinstate the post in the White House, the recommendations were clearly aimed at his successor.

Thus, President Gerald Ford has inherited an unhappy scientific community, a strong feeling among the scientific establishment that science should be reinstated in the White House, and a host of problems such as the energy crisis, and food shortages which will require large injections of science and technology.

Clearly, the science policy apparatus is not going to be one of Ford's immediate concerns, and little change can be expected in the short term. Ford himself has said that his immediate concern is to curb inflation, however, and that, indirectly could have a bearing on science.

Ford is a self-confessed fiscal conservative, believing in balanced budgets and decreased federal expenditures. Since only a relatively small proportion of the federal budget can be decreased—the vast majority of the budget is for such items as salaries, pensions and welfare payments which cannot be tinkered with—and since science expenditures mostly fall into the controllable category, the pressure on the science budget is unlikely to decrease under President Ford.

Two approaches to scientific aid for disaster areas

Scientists and nonscientists alike are becoming increasingly aware of the need for science to be 'relevant'. In human terms, perhaps the most important applications of science are to the problems facing the less developed parts of the world, and the need for relevant science can be seen most clearly at times of crisis—such as the recent floods in Bangladesh. But how effectively is science being used in such situations? John Gribbin and John Wilson have been looking at two contrasting approaches to these problems.

ESTABLISHED charitable organisations such as Oxfam are making increasing use of science and technology in their work; it is becoming accepted that provision of food to survivors of a disaster is no more than a temporary solution to any problem, and that famine and disease can only be prevented by more fundamental help. But still, only a tiny fraction of Oxfam's income, for example, is spent on research — although that small budget seems to be used remarkably effectively.

Among projects Oxfam is working on are:

- A completely new sewage disposal system which can be flown straight to disaster areas and is, according to Oxfam, cheap, simple and easy to erect.
- A new building technique to provide rapid emergency housing after an earthquake, flood or other catastrophe.
- An investigation of bicycle 'pedal power' in the poorer countries.

These are all projects with a sound scientific pedigree. The sewage scheme, for example, required fundamental research on the biology of the cholera vibrio. But money allocated by Oxfam to such research was only £3,000 in 1973, out of a total 'income' from public donations of £4.2 million, more than 80% of which was spent overseas.

The Deputy Director of Oxfam, Mr Guy Stringer, explains that the best use is made of this £3,000 by using Oxfam money only to prime the financial pump of a project. Once a plan has shown potential, Oxfam seeks help from other sources. This falls into line with the organisation's avowed policy

of spending as much of the public's money as possible 'over there' rather than on research at home. And the people and firms that Oxfam approaches are eager to help. "They have never refused us", says Mr Stringer.

Oxfam undoubtedly benefits enormously from this goodwill; but part of its success must surely lie in the simple direct approach which it adopts. The charity first became interested in sanitation schemes as a result of its experience of the refugee camps in Bengal during the Indo-Pakistan conflict of 1971. Oxfam workers there realised that most of the relief effort was being spent on the treatment of diseases arising from the insanitary conditions within the camps. Even so, this preventive medicine was often ineffective—particularly against cholera.

At first Oxfam simply tried to contain the excrement and other wastes of the refugees. It found that one possible container (a 30,000 gallon collapsible fuel tank belonging to the RAF) provided almost instant anaerobic conditions. From that discovery sprang the idea of destroying the cholera and dysentery bacteria anaerobically.

As no one knew the viability of *Vibrio cholerae* in anaerobic sewage, Oxfam asked Mr Barry Lloyd of the University of Surrey to find out. To keep costs as low as possible, Mr Lloyd presented the project to two final year students as the topic for their degree theses. Although each thesis 'cost' about £800, Oxfam paid only a tenth of this for the results.

The survival of the vibrios was found to depend on the temperature of the sludge and the proportions of solid matter in it. Vibrios were usually eliminated after 7 days at 37°C but at 25°C took 12 days to disappear. Once Oxfam had an estimate of how long the sewage should be retained in anaerobic conditions, it was able to seek the advice of the Water Pollution Research Laboratory at Stevenage, and the University of Loughborough on the general layout and hydraulics of the unit. The Plastics Research Group at the Atomic Energy Research Establishment, Harwell, designed a mould for a cheap stackable plastic squatting unit—the Asian equivalent of a toilet seat.

Mr Jim Howard, Oxfam's Industries Officer, emphasises the importance of

this squatting unit. Produced now for a matter of shillings, they replace heavy vitreous china items that cost about £50.

The sanitation package on which Oxfam has now decided consists of two or three large butyl rubber tanks holding some 4,500 gallons each, connected in series to a group of 20 squatting units. During October, a team from Oxfam will be taking an example of each unit to Bangladesh. With the cooperation of the Cholera Research Laboratories in Dacca, the two-bag unit will be tested on hospital effluent known to be rich in cholera bacteria and the three-bag system will be set up in a Bihari refugee camp to assess its impact on an existing community.

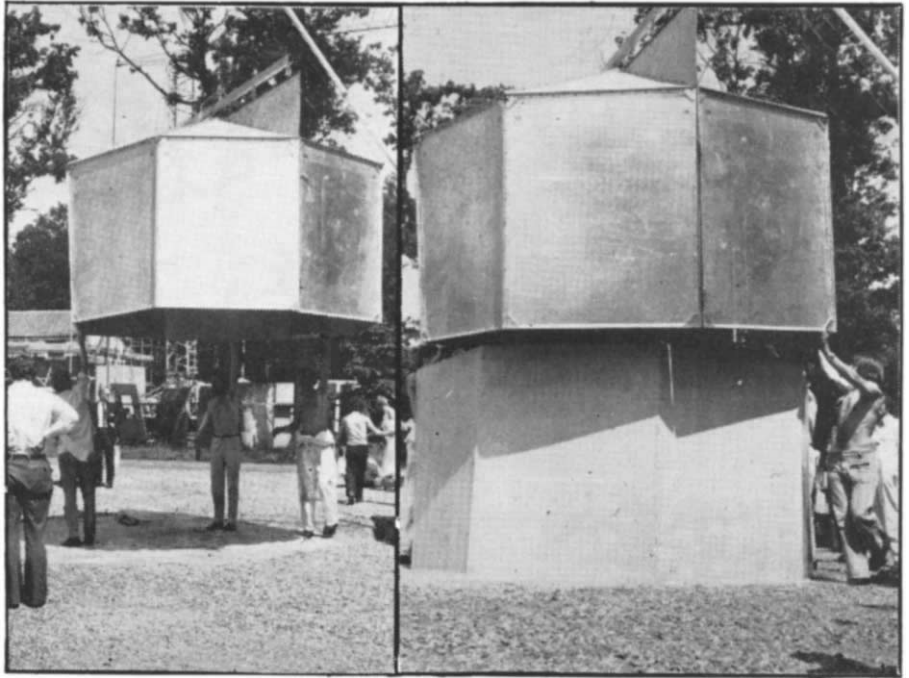
Even including the cost of this visit, Oxfam says it has spent less than £1,500 on the entire sewage project. Once the scheme was underway the Leverhulme Trust provided a grant of £19,000 and the British Government gave £6,500. The charity holds British, United States and Canadian patents on the system so it may still recoup what little it spent. With the addition of antifreeze the technique might be adapted for use in the Arctic or Antarctic.

The sanitation scheme is probably the most ambitious technical project that Oxfam have tackled and it typifies the organisation's direct approach to disaster relief. In the same vein, Oxfam is pioneering a building technique which it hopes will provide warm weather-proof shelters quickly and cheaply. Polyurethane foam is sprayed on to the inside of a lightweight aluminium mould. When the foam hardens, the mould is removed leaving a house with approximately 70 square feet of living space.

The mobile factory to spray the foam costs about £4,500 and Oxfam claims that the chemicals needed for one house may be bought for £30. Polystyrene granules are cheaper—and are rather more fire resistant—but the apparatus needed to steam them into place is six or seven times as expensive as the polyurethane apparatus. In September, Oxfam will be training two volunteer teams in the spray building technique.

In many underdeveloped countries a great proportion of the labour force—as much as 15% in some cases according to Mr Howard—spends its time moving water for irrigation. Oxfam is now trying to develop a portable

*Native huts, Oxfam style.
An aluminium shell is lowered into
place (left) and removed after serving
as a mould (right).*



water pump that could be powered by pedals in the same way as a bicycle. It is also investigating this use of pedal power to drive simple winnowing or grinding machines. With Mr Stuart Wilson, of the University of Oxford, it is developing a superior rickshaw, and a four wheel drive pedal platform or 'Pedalrover' which could carry half a ton across country; Zambia has already expressed an interest in this idea.

Oxfam does not always design its schemes for its own use. In 1971 it began a project to turn Britain's surplus potato crop and skimmed milk into a balanced emergency diet. Although Oxfam persuaded Cadbury Schweppes to let it use their powdered potato production lines at cost price it still spent £6,250 of its own money. Mr Howard feels that since Oxfam's new food is now accepted by the World Food Programme, it is up to the government to use the recipe when and if a surplus occurs again.

But Oxfam is not without its critics. Some people have expressed concern, for example, that any temporary housing provided after a disaster might remain occupied when conditions returned to normal, degenerating into instant slums. Oxfam admits that further work is needed to determine an optimum spacing of such units to minimise the fire hazard while housing as many people as possible—but it is fundamental to their philosophy that such housing is needed, although a case can be made that in many places, such as Bangladesh, the local population already possesses both the skills and the material needed for the rapid erection of cheap lightweight shelters.

At a more fundamental level, questions might be asked about whether such organisations as Oxfam are in fact the best bodies to organise scientific aid for disaster situations. The point is debatable; but it is true that Oxfam is not run by scientists, and that its scientific activities are still very much an appendage to the main work. So it is interesting that a group of London-based scientists is trying just the opposite approach. The London Technical Group (LTG) is a group of scientists from various disciplines which is looking at problems of disaster relief solely from the scientific and technological point of view.

The most widely available tangible product from the group so far is an

annotated bibliography of papers relating to *Disaster Technology* (LTG, 55 Evelyn Gardens, London; 1974). This typifies one aspect of the group's activities to act as a clearing house for relevant information and, hopefully, to ensure that such information does not disappear from the general awareness. Members of the group say that they have been astonished at how often 'new' ideas turn out to have been presaged years or even decades ago. They cite the example of the compression effects which cause internal injuries in victims pulled from collapsed buildings and have been "discovered" after almost every major earthquake affecting built up areas. In fact, they say, these problems were thoroughly investigated more than three decades ago, when many people were trapped in collapsing buildings during the mass bombing of the Second World War.

But the LTG is not just concerned with collecting and distributing information. Members of the group (now some 30 strong) see the LTG as something of a centre of expertise in terms of field experience of disaster and famine situations, eager to hire out their skills to anyone who can use them.

The opportunity for scientists with such expertise to meet regularly and discuss problems provides the third string to LTG's bow, as a 'disaster think tank'. Such ideas as mixing dried skim milk with oil to form a drinkable and nutritious emulsion (with the addition of appropriate substances to make it palatable) and an emphasis on rugged simplicity for all field equipment emerge from these meetings. Individual field trips made by members of the LTG have provided the essential training in

basics which encourages the group to think that it now has the experience to, say, carry out surveys of nutritional problems on behalf of governments or other bodies, who would put up the money and collect the results on a contractual basis. These trips also, of course, provide valuable information in their own right. Mr John Rivers, a member of the LTG, has just returned from Ethiopia, where he carried out a nutritional survey with Dr John Seamen as part of a UNICEF project, partially funded (to the tune of £1,000) by Oxfam. It seems from this work that the general feeling that protein deficiency may not be the greatest nutritional problem in famine areas may well be correct; the normal food of the nomads in Ethiopia includes milk, cereal and a little meat. In the present famine situation they are forced to eat other foods, such as beans. But there is no evidence that the quality of these foods is inadequate, whatever the problems of finding enough food; and this means that any emphasis on protein supplements as a high priority in famine relief is wrong, at least in this case.

It remains to be seen whether any charity or other organisation will jump at the opportunity of hiring the LTG team of scientific specialists as a group to do this kind of work, or whether the LTG will continue simply as a collection of concerned scientists devoting their spare time to efforts aimed at improving the way science is used to combat disasters. The approach they advocate is, however, worthy of serious consideration if only as a reminder that there are alternatives to the existing systems which have, by the nature of things, become 'the establishment'.

international news

CITING a sheaf of evidence which indicates that the pesticide dieldrin is highly carcinogenic to mice and rats, and that it now resides in the tissues of virtually every man, woman and child in the United States, the Environmental Protection Agency (EPA) last week abruptly halted further production and recommended that it be phased out of use as rapidly as possible.

The decision is the latest, and most significant, milestone in a four-year legal battle fought by a Washington-based environmentalist organisation to get dieldrin off the market, but it is far from the final word on the matter. Officials of the Shell Chemical Company, the sole manufacturer of the pesticide, erupted with indignation at the EPA's action, charged that "there is no evidence whatsoever to associate this chemical with cancer in man", and demanded a public hearing to state its case. The hearing is now going on, and a final decision is expected by the end of August.

If all that sounds familiar, it is. The history of the battle against dieldrin bears a strong resemblance to the long and bitter fight against DDT, although according to opponents of the pesticide the case against dieldrin is even more cut-and-dried than that against DDT. In both cases, the central issue is the ubiquity of the pesticide in the environment and the possibility that it may cause cancer in man.

Although it is a pesticide in its own right, dieldrin is also the breakdown product of the chlorinated hydrocarbon pesticide aldrin; the two agents are considered together in the EPA decision.

Firm favourites in the eyes of growers of corn and citrus fruits, the pesticides are used chiefly to control soil insects. They are applied directly to the soil in the spring, essentially as an insurance against the possibility of an outbreak of soil pests later in the year. Their longevity is thus a prime requisite, since they may have to kill insects weeks after they are put into the soil and, according to Shell and the United States Department of Agriculture which staunchly defends the use of aldrin and dieldrin, no other pesticide can remain active for long enough to do the job.

But the persistence of these pesticides is a mixed blessing, for dieldrin now contaminates many foods and is stored in high concentrations in human fatty tissues. According to a survey carried

EPA halts dieldrin production

by Colin Norman, Washington

out by the Food and Drug Administration last year, for example, measurable amounts of the pesticide were found in 83% of all dairy products, 88% of all garden fruits, 96% of all meat, fish and poultry, and in 12 to 14% of samples of grain, potatoes, fruit and some other foods. Moreover, in 1971, the EPA found that 99.5% of human tissue samples taken during autopsy or therapeutic surgery contained amounts of dieldrin averaging 0.29 parts per million.

There is thus no denying that man is constantly exposed to the pesticide. But there is considerable debate about whether this exposure carries a risk of cancer, and it is on that point that the case really hinges.

The EPA issued its order last week chiefly on the basis of studies which have shown that dieldrin "definitely causes significant increases of tumours in two and possibly three strains of mice tested", and that the pesticide has also raised tumours in two different strains of rats. The tumours, which "have been diagnosed unequivocally as malignant", appear in the liver, lungs, lymphoid tissue, thyroid, uterus and mammary glands, and have resulted from feeding tests involving doses as low as 0.1 parts per million. The EPA also cited evidence that exposure to dieldrin for periods as brief as a few weeks has caused significant carcinogenic effects in test animals.

The case against dieldrin was brought out during weeks of public hearings conducted by the EPA to determine whether or not the pesticide should be removed from the market. Those hearings are not expected to be completed until late this year or even early next year, but while they have been going on, Shell has been free to continue manufacturing both aldrin and dieldrin.

In April, however, EPA officials considered that the evidence against the pesticides—which came from studies conducted at the Food and Drug Administration, the National Cancer Institute and in Shell's own laboratories—was so compelling that they contacted

Shell officials and asked them to halt production of aldrin and dieldrin voluntarily until the public hearings have been completed. But Shell officials maintained that there is no evidence that the pesticides are harmful to man, declined to accede to the EPA's request and announced that they would begin producing next year's batches of aldrin and dieldrin on September 1. Thus the EPA moved last week to force Shell to stop manufacturing the chemicals, at least until doubts about their safety are cleared up.

For its part, Shell maintains that the animal feeding studies have little applicability to man, and that the safety of aldrin and dieldrin is assured by the fact that workers in factories manufacturing the pesticides have shown no signs of developing cancers. Lawyers for Shell have yet to present their case in full at the public hearings, and are annoyed that the EPA is trying to halt production of the chemicals before all the facts have been argued.

If Shell is allowed to go ahead with its production in September, millions of pounds of the chemicals will be distributed around the United States, and if the public hearings eventually conclude that the pesticides do pose a serious health risk, it would be virtually impossible to call all supplies in. Thus, the EPA is trying to ensure that if it bans the pesticides at the end of the public hearings, they will not continue in use for another year. □

More radiation protection

MICROWAVES and lasers are to be the first two types of non-ionising electromagnetic radiation to be taken under the wing of the British National Radiation Protection Board (NRPB) under an order extending its functions.

Since its inception as a result of the National Radiological Protection Act of 1970, the board has confined its advisory and research services to the fields of ionising radiation. Although it has no statutory powers, it advises the government and other responsible authorities on standards and safeguards against radiation hazards.

The recent order, by the Secretary of State for Health and Social Services, came into effect on August 1 and extends the functions of the board to cover potentially all electromagnetic

radiation. Its interest is being concentrated on lasers and microwaves in the first instance, say the board, not because these pose any widespread health hazard but because their use has increased enormously in the past few years and some national authoritative reference is now desirable.

Already, there is a generally agreed safety limit for continuous microwave exposure of not more than 10 mW cm⁻² average power density. This is rigorously observed by present users of radar, one of the main commercial applications of microwaves in Britain.

But there is another growing commercial use of microwaves, in quick microwave ovens which are not yet big business in Britain but whose use will probably increase. The board is setting up an advisory and research service at its Leeds centre, where ovens can be tested for radiation leakage. The second part of the NRPB's extended brief is the use of lasers. □

Taxing your sabbatical

UNTIL the end of the tax year on April 5, 1974, visitors to Britain on sabbatical leave were taxed by the British authorities on a so-called 'remittance basis'. The sum of money actually brought into the country was the base for taxation and in the six years before a sabbatical became due, many academics had learnt how to prevent most, if not all of their income of the seventh year (assuming it came from their permanent institution) from entering Britain. Legislation that has just been passed radically alters the tax position.

It is assumed in what follows that the work done in Britain is not entirely unconnected with normal duties of employment in the home country—an assumption which the tax officer is almost certain to make if the permanent institution is paying.

In the case of visitors from the United States, half of their pay will be liable to tax in Britain for any tax year in which they are in the country for 183 days or more; these days need not necessarily be consecutive. Any tax year in which visits do not add up to 183 days is not considered. A double taxation convention applies with the United States and so credit for British tax paid can be claimed against the United States tax on the same income. If a visitor becomes liable for tax through being in Britain for six months or more, he is entitled to claim full personal allowances.

Similar arrangements are in force with many other countries but tax authorities point out that it should not be assumed that arrangements are

identical and detailed information should be sought from embassies.

This change in the tax situation has been widely asserted to drive potential visitors away from Britain. In fact the most likely to suffer from it are those who have been able to find a way to avoid remitting income to this country, say by borrowing for their visit. Anyone who has had, of economic necessity, to bring his income with him, has previously had to pay tax on the full amount remitted in tax years in which he has been here for six months or more. Now he pays tax on only half that amount. □

Business: CEBG awash with capacity

by Roger Woodham

WHATEVER the Central Electricity Generating Board's financial position may be—it made a loss of £87.4 million in 1972–73—the board evidently stands no risk of running short of generating capacity as it did in the winter of 1969–70. At that time the CEBG's maximum output capacity was some 46,000 MW and the maximum demand to be met was about 38,000 MW, but for a string of reasons that were investigated by the Select Committee on Science and Technology insufficient capacity was actually available when it came to the crunch and the result was a series of voltage reductions—'brown outs'.

The situation as of March 1974 was much different in that the maximum output capacity had soared to 58,000 MW whereas the maximum demand that winter had been about 40,000 MW. The latter figure had not changed appreciably for three years (*CEBG Statistical Yearbook 1973–74*).

The inescapable conclusion is that the CEBG has an unrealistic amount of surplus capacity which it busily added to in 1973–74 at a cost of £189 million, representing 1,600 MW. And the plant now under construction which should be available by the end of 1978 will provide a further 12,000 MW, bringing the total to perhaps 67,000 MW if allowance is made for the demise of old plant. If the growth of electricity demand were about 3% a year in the next few years, the maximum call on the CEBG's services would only rise to some 45,000 MW by 1978. At that stage the board would be capable of meeting a peak demand of half as much again. Naturally some of that 'overkill' must be regarded as a provision for plant temporarily out of service, but the margin is different altogether from the 21% which proved to be insufficient—just—in exceptional circumstances in 1969–70. In 1966–67 the CEBG was getting by without bother on a margin of about 12%. □

Qualified approval for Aspartame

by Colin Norman, Washington

THE United States Food and Drug Administration (FDA) last week gave its approval for a new artificial sweetener to be used in a variety of products. Called Aspartame, it is 180 times sweeter than sugar and its official debut is significant since it comes right in the middle of an investigation of the safety of saccharin—the only other artificial sweetener on the market in the United States—which is suspected of causing bladder tumours in mice.

Aspartame loses its sweetness on prolonged cooking, however, and thus it has only been approved for such uses as sweetening tea and coffee, adding to breakfast cereals and for sweetening puddings, gelatins and artificial cream. So far, it has not been approved for use in so-called diet drinks, and it will therefore not challenge saccharin in its largest market. But FDA officials suggest privately that soft drink manufacturers will soon petition the government to allow them to use Aspartame.

So far, few people have expressed doubts about the safety of Aspartame, chiefly because it is broken down in the body into two essential amino acids, L-aspartic acid and L-phenylalanine. Furthermore, FDA's approval was based on the results of feeding studies which involved dogs and rats for two years and a number of rats which were exposed to Aspartame *in utero* and throughout their lifetimes. The studies gave no indication of tumorigenicity, and suggested that at least 2 grams per kilogram of body weight are required before any toxic effects are evident. That level is more than 100 times greater than the likely average daily intake of the sweetener in foods for which it has so far been approved.

The FDA's decision will be closely studied in several other countries—including Britain—where applications have been made by G. D. Searle and Co., the manufacturer of Aspartame, for permission to market the sweetener. Its importance in the United States is that it gives the FDA more flexibility in dealing with two particularly sticky problems. First, the FDA has been forced to re-evaluate its decision to ban cyclamates from the market on the basis of suspected carcinogenicity (a decision is expected early next year) and, second, saccharin is now under investigation and the FDA will probably have to decide its fate within the next couple of months. Before Aspartame came along, the FDA was facing the problem of perhaps removing from the market all artificial sweeteners, but now at least it has a third product to insert into the cost-benefit equation. □

correspondence

Victimisation

SIR,—What Professor Burhop in effect is saying (*Nature*, August 9) is that the "Declaration on the Rights of Scientists", made by the World Federation of Scientific Workers in 1969, is really no more than an expression of pious hopes, since there is nothing that can be done about victimisation by governments, left, right, or centre, if the governments responsible are themselves disinclined to cooperate.

As President of the W.F.S.W., Professor Burhop is no doubt well placed to judge of this. But when he goes on to say that "... the close contacts maintained between our affiliated organisations in different countries are beneficial to scientists and for science itself", one wonders whether there might not be long-term benefits for science and scientists in particular countries, if the W.F.S.W. were to make it clear that although "Our aim must be to strengthen these contacts", that policy would have to be applied selectively, where there was reason to believe that the "Declaration" was not being adhered to by the governments or affiliated organisations concerned.

Yours faithfully,

C. B. GOODHART

Cambridge, UK

Plasmid engineering

SIR,—The appeal by a committee of the National Academy of Sciences for restraint with respect to certain types of genetic experiment (*Nature*, July 19) deserves the generally favourable response accorded it (*Nature*, July 26). The potential hazards associated with the creation of artificial recombinant DNA molecules are clearly outlined in the committee's statement. However, there is an unfortunate lack of clarity in defining the types of experiment which it is recommended should be deferred. The statement concerning experiments of type I is confusing. This is particularly so if one does not persevere to the last three lines of the inordinately long sentence which excludes "plasmids containing such combinations of antibiotic resistance determinants (which) already exist in nature." The hazards associated with these latter plasmids are not potential but have already been amply demonstrated and are well understood, as explained by Dr Anderson. The NAS committee's recommendation is concerned with unnatural recombinant plasmids and it would be unfortunate if, as seems likely from parts of Dr Anderson's comment,

the lack of clarity in the definition of experiments of type I led to the inclusion in the recommended ban of experiments concerned, not with future potential hazards, but with those already with us and in need of attention.

Much work is in progress, including my own, with the specific objective of seeking means for the effective elimination of plasmids from bacteria. It would be against the spirit of the NAS committee's aims if misinterpretation of the nature of experiments of type I brought to a halt, even temporarily, experiments carrying no potential hazard from new recombinant DNA molecules, but concerned with already existing hazards in clinical practice and animal husbandry.

Yours faithfully,

G. R. BARKER

Manchester, UK

Scientists don't move

SIR,—Your editorial of July 26 on Sir Hermann Bondi's report on the interchange of scientists was very critical of the lack of mobility of scientists, and could scarcely believe that they are inhibited from changing jobs because of the difficulty of moving house. My recent experiences may help you to understand the problems.

Last December I moved from a civil service post in Farnborough to a research fellowship at Leicester University. It took the Civil Service Department over four months to determine the transfer value of my pension, but this was certainly no obstacle to me. Indeed I think that the preservation or transferability of pension rights is of little concern to the young scientist contemplating a move. My new employers seem to be unusually generous by contemporary standards in that they reimbursed a large part of my "removal expenses" and also provided a small second mortgage loan at low interest rate. Even so we have been faced with unreimbursable expenses in the present dormant housing market of more than £2,000—probably nearer £3,000 by the time the flat is sold.

My present contract lasts for only three years, and, with the present tendency of university science departments to contract, it is entirely possible that I shall have to move again in 2½ years' time, with, no doubt, a repetition of these expenses.

In North America or any other country of Western Europe, people

in our position find it quite natural to rent a house for a few years; here it is impossible to find unfurnished accommodation. Even worse, the 1974 Rent Act, by giving security of tenure to furnished tenants as well, has made us abandon any idea of letting our empty flat in this way. It seems to be the policy of both major parties that every family should either own its own home or be a council tenant, but there can be little doubt of the severe effects of this policy on the mobility of all labour, and not just scientists.

While I have no regrets at the change in my working environment which I have bought at the price, perhaps, of two years' salary, I feel unable to criticize the many scientists who do not choose to follow my example. They too may count the cost, and they may feel that boldness in pushing back frontiers is not incompatible with financial solvency.

Yours faithfully,

C. G. PAGE

Leicester, UK

Collecting egg-whites

W. R. P. BOURNE (*Nature*, 249, 793; 1974), seems determined to continue to hound Professor Charles Sibley for his comparatively minor indiscretion (with the connivance of others) in obtaining the eggs of certain birds unlawfully.

Professor Sibley is not, and never has been, an egg collector in the accepted sense as the title of Bourne's article suggests. Indeed his interest in eggs has been confined to the analysis of egg-white protein and the taxonomic value of such analysis as far as avian relationships are concerned.

Whatever the final results of Professor Sibley's researches may be, it is certain that they will be studied by all who are interested in the many unsatisfactory aspects of the classification of birds, typified, for instance, by the Babblers (Timaliidae), for too long the despair of orthodox systematists.

R. WAGSTAFFE

Bluntisham, Huntingdon, UK

How many deer?

SIR,—How many deer have been 'sacrificed' (which is, I believe, the correct scientific term) in order to provide linings for "attractive space-saving files for your *Nature* ... beautifully labelled"?

Yours faithfully,

Hove, UK

B. JOVE

news and views

Current work with the voltage clamp

THE present view of the ionic basis of the action potential in nerve cells depends largely on the classic voltage-clamp experiments of Hodgkin and Huxley, published in 1952 (*J. Physiol.*, **116**, 449). Although it is remarkable how well this analysis has withstood the test of time, recent work has shown that the changes in potassium permeability are more complex than previously realised and that Ca^{2+} ions may play an important part in the inward current in many neurones.

The action potential of nerve cells is essentially a transient reversal of the voltage across the cell membrane. Hodgkin and Huxley showed that it is generated by brief, sequential increases in the membrane permeability first to Na^+ ions and then to K^+ ions. They used a voltage-clamp system to control the membrane potential of a squid giant axon, and were able to measure the currents flowing across the membrane. Following a large depolarisation the current first flowed inwards, and then rapidly reversed and was maintained in an outward direction, as shown in the figure (a). This membrane current could be separated into an inward one carried by Na^+ , and an outward one carried by K^+ . The Na permeability was rapidly switched on, or activated, and then inactivated after a short delay. The K permeability was activated only after a short delay, but was apparently not inactivated. It remained constant until the depolarisation ended.

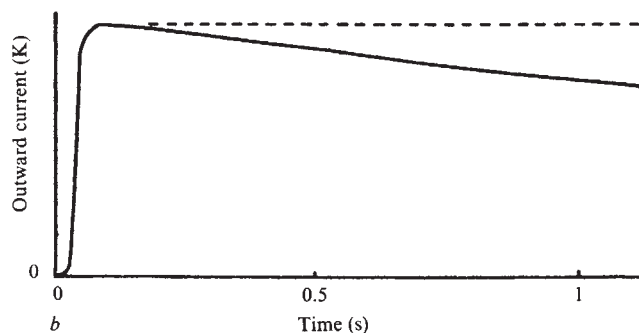
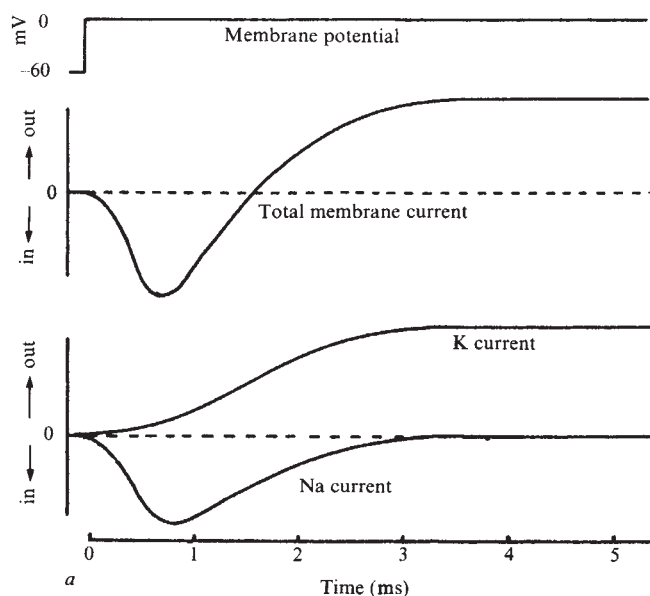
Within a few years of this elegant work it was shown in several preparations, particularly in mollusc nerve cell bodies, that the removal of external Na does not block action potentials. Voltage-clamp studies have now demonstrated that the inward current can be carried by Ca^{2+} as well as, or instead of, Na^+ . Some Ca^{2+} may pass through the standard Na permeability system (Baker, Hodgkin and Ridgway, *J. Physiol.*, **218**, 709; 1971), but it is not yet clear whether there is a separate permeability system which handles most of the Ca current. Geduldig and Greuner (*J. Physiol.*, **211**, 217; 1970) reported that the activation properties of Na and Ca permeabilities in *Aplysia* neurones are different, suggesting separate systems. Kostyuk and colleagues (*Pflügers Arch. ges. Physiol.*, **348**, 83; 1974) and Standen (*Nature*, **250**, 340; 1974), both using snail neurones, have recently presented evidence, however, that the two ions use either the same system or, possibly, parallel ones with identical kinetics.

Even more involved than these complications of the early inward current system are those concerning the K permeability. As well as the well-established (or slow) outward K current, Neher (*J. gen. Physiol.*, **58**, 36; 1971) found a fast outward K current in snail neurones with kinetics similar to those of the inward current. Connor and Stevens (*J. Physiol.*, **213**, 31; 1971) also separated the potassium current that they measured in dorid neurones into a fast component that had both an activation and inactivation mechanism and the more usual slow component. This fast K current seems to be particularly important in controlling pacemaker activity, and may play an important part in repetitive firing.

Another aspect of the K current which has recently

come to light is the observation of a K inactivation mechanism. Whereas Hodgkin and Huxley's K current was maintained indefinitely during a depolarising step, several recent experiments have shown that it can decline (see figure b). Connor and Stevens found that the slow component of the K current inactivated with a time constant of several seconds. They also showed that it was a distinct phenomenon (that is, not a simple inactivation of the fast K current) by demonstrating differences in the voltage-dependent characteristics and the responses to TEA. Many examples of K inactivation have now been reported: to cite only two of them; Leicht, Meves and Wellhoner (*Pflügers Arch. ges. Physiol.*, **323**, 63; 1971) measured an inactivation of the delayed K current in snail neurones with time constants of 0.5–1.75 s and Ehrenstein and Gilbert (*Biophys. J.*, **6**, 553; 1966) found a similar inactivation in squid axons with a time constant of 11 s. Such slow processes, although obviously of no importance in the generation of single action potentials, could play an important part in the long-term firing characteristics of the cell.

One difficulty with the voltage-clamp technique is that it only measures the net membrane current. It is not always easy to determine whether changes in the recorded current



Hypothetical voltage-clamp records of voltage or current against time.

result from changes in an inward or an outward component. Although it is relatively easy to establish that the inward current is carried by Na^+ or Ca^{2+} ions by removing them from the external solution, it is difficult to show directly that K^+ ions carry the outward current. An elegant way of doing this was recently reported by Neher and Lux (*J. gen. Physiol.*, **61**, 385; 1973). They used a K^+ -sensitive microelectrode to measure changes in K^+ outside snail neurones, and showed that the total amount of K^+ released by the cell during a clamp pulse was in good agreement with that calculated assuming that the outward current was carried only by K^+ ions. Lux and Eckert (see page 574 of this issue of *Nature*) have now applied this ingenious technique to a study of apparent K inactivation. When the outward current was reduced by procedures expected to produce K inactivation, the actual release of K^+ , as determined by the K^+ -sensitive microelectrodes, was not equally reduced. They conclude that there must be a slow inward current as well as the normal slow outward current. Part of the apparent K inactivation was the result of this unsuspected inward current, which would make the outward current as recorded by the clamp smaller than the true outward current. The ion or ions carrying this slow inward current are so far unidentified, but are presumably Na or Ca.

ROGER C. THOMAS
L. DONALD PARTRIDGE

Germline antibody genes

THE genetic basis for antibody diversity is a subject of continuing interest though the argument between germline and somatic models of antibody diversity has abated recently. The large number of different amino acid sequences so far determined for V regions of mouse Kappa chains, point to there being at least a hundred V_{Kappa} genes and there is probably a similar minimum number of V_{H} genes. The question as to whether somatic processes are used to increase the diversity encoded in the multiple germline V genes remains open. Now the major effort is aimed at defining the way in which the V and C genes are arranged in a chromosome and the way in which they function.

At present it is thought that the genes which code for H chains (V_{H}) are present on the same chromosome with the set of about ten C_{H} genes, one C_{H} gene for each class of antibody. In this model any V_{H} gene can be paired with any C_{H} gene to give a gene pair coding for a single heavy chain. Although there is considerable evidence to support this scheme, at present it is only an outline of what must be a fascinating genetic process. The C genes for both light and heavy chains map as single genes in a Mendelian fashion. Similar mapping of individual V genes has been limited by the lack of suitable phenotypic markers. Recently individual antibodies have been characterised by either their idiotype (characteristic antigenic determinants defined by antisera raised against the antibody under study) fine specificity (definition of an antibody combining site by the quantitation of cross reactions) or spectotype (characteristic isoelectric focusing spectrum of an antibody). Using these genetic markers six V_{H} genes have been identified in the mouse and a search is now being made for recombinants so that a map of the V_{H} region of the chromosome can be drawn. Each of these V_{H} genes, in agreement with the model I have mentioned, has been found to be closely linked to the C_{H} loci.

Eichmann, Tung and Nisonoff reported in last week's edition of *Nature* (250, 509–511; 1974) strong evidence for a recombinational event separating two of the known V_{H} genes *ARS* and *A5A*. These genes had been identified by

the idiotype present on A/J strain mouse antibodies directed against *p*-azophenylarsonate (*ars*) and group A streptococcal carbohydrate respectively. A/J mice can make a variety of different antibodies directed against each of these two antigens. The initial response of A/J mice to *ars* always includes an antibody (or a family of antibodies) carrying the *ARS* idiotype determinant. The *A5A* idiotype has been found to be associated with a particular antibody spectotype and cells producing that antibody have been cloned *in vivo* (*Eur. J. Immunol.*, **2**, 301; 1972). The inheritance of *A5A* in the C_{H} linkage group was shown by Eichmann and Berek (*Euro. J. Immunol.*, **3**, 599; 1973) who mated A/J mice against BALB/c mice, a strain which efficiently makes antibody against group A carbohydrate but has never shown that *A5A* idiotype; the F_1 progeny were backcrossed against BALB/c. Of the backcross offspring 16/29 were homozygous for the BALB/c C_{H} genes; all but one of those 16 were, as expected, negative for the *A5A* idiotype on their anti-group A carbohydrate antibody. The odd mouse BB♂7 showed a recombinant phenotype with the *A5A* gene apparently present in the BALB/c C_{H} linkage group.

In Eichmann *et al.*'s latest study, BB♂7 has been again backcrossed against BALB/c and the progeny support the idea that a new linkage of *A5A* to the BALB/c C_{H} loci has occurred as the result of a recombinational event. Many of the progeny of BB♂7 have been tested for the *ARS* idiotype. In this respect the backcrosses behave just like the parental BALB/c mice and make anti-*ars* lacking the *ARS* idiotype. The one recombinational event observed among the three genes under study is consistent with a simple linear map *A5A*—X—*ARS*— C_{H} —where X shows the position of the crossover. Further crossovers are needed in order to estimate map distances between these three genes.

The occurrence of a recombinant in only sixteen progeny in which it could be detected is either a very fortuitous event or else it is indicative of a large map distance between *A5A* and the other two genes. One map distance in the H chain linkage group of the mouse was reported by Riblet at the workshop on immunoglobulin variable region genetics (Bethesda, Maryland, March 25 and 26, 1974). Riblet studied the *DEX* gene which is characterised by an idiotype specificity found on two BALB/c myeloma antibodies binding α -1,3 dextran. Only two recombinational events were observed in a total of 530 crosses in which a recombination could have been detected. At the time of writing their article, Eichmann and his colleagues had screened 73 meaningful crosses and BB♂7 remains the only recombinant. The next recombinant is keenly anticipated since the measurement of the distance between *A5A* and *ARS* will be indicative of the number of V genes which might lie between these markers.

ALAN R. WILLIAMSON

Reappearance of hexosaminidase A

SINCE it became possible to fuse somatic cells using Sendai virus people have been investigating the complementation (supply of the other's needs by each parental cell) of closely similar genetic defects. Complementation, in hybrids and heterokaryons (the multinucleated cells produced by fusion) has provided useful information about recessive mutants, in cases, for example, where the precise metabolic lesion is not known and genetic heterogeneity has been neatly demonstrated (see, for example, Kao *et al.*, *Science*, **164**, 312; 1969; de Weerd-Kastelein *et al.*, *Nature*

new Biol., **238**, 80; 1972). If different genes are defective in each parent then the normal gene product from the opposite parent in each case will be present in the hybrid with comitant 'normal' or 'heterozygote' phenotype—namely intergenic complementation. If on the other hand the two parental cell lines have a mutation at the same gene locus, complementation does not usually occur; the hybrid line also shows the defect.

Different mutations at the same locus, however, can it seems sometimes complement each other (interallelic complementation) as the result of the formation of functional proteins (enzymes) made up of two different types of defective subunits, the variant gene products from each parent. Although interallelic and intergenic complementation are indistinguishable when the primary product of the relevant gene is not identified, in cases where an enzyme deficiency has been observed in the parental cells, the newly synthesised 'hybrid' enzyme produced as a result of interallelic complementation would probably show differences in property from the normal enzyme.

Genetic complementation has now been pleasingly demonstrated in heterokaryons made by fusing skin fibroblasts from patients with Tay-Sachs and Sandhoff's disease, two clinically very similar diseases characterised by the excessive accumulation of the ganglioside GM₂ in the brain. In the tissues of patients with Tay-Sachs disease the enzyme N-acetyl β -D hexosaminidase A is absent and in Sandhoff's disease both major forms of N-acetyl β -D hexosaminidase (A and B) are deficient. What Thomas *et al.* have done (see page 580 of this edition of *Nature*) is to demonstrate that hexosaminidase A (as well as B) is synthesised in the heterokaryons. Unfortunately, this finding does little to quell the dispute about the relationship of the hexosaminidase isozymes, because it can be explained by all the current models.

Until the past week or two only two main models had been considered in the literature. The first is that B, the precursor enzyme, determined by locus 1, is enzymatically converted secondarily (by the product of locus 2) into A; and the second and perhaps most favoured theory is that A and B possess similar and dissimilar polypeptide subunits ($A=(\alpha\beta)_n$; $B=(\beta\beta)_n$ or $(\beta\gamma)_n$) α being deficient in Tay-Sachs and β in Sandhoff's disease (see, for example, Carroll and Robinson, *Biochem. J.*, **137**, 217; 1974; Srivastava and Beutler, *J. biol. Chem.*, **249**, 2054; 1974). Both of these models invoke at least two separate gene loci, a different one being affected in each

disease, and if either is right the synthesis *de novo* of hexosaminidase A in heterokaryons and hybrids would be predicted. If, however, only one gene were involved, the finding could still be explained by the formation of a hybrid enzyme by interallelic complementation. Yet another lengthy article on the interrelationship of the hexosaminidases has just appeared in the *Journal of Biological Chemistry* (Tallman *et al.*, **249**, 3489; 1974) and these authors conclude that the A and B enzymes are conformational isomers—a one gene model. They propose that the mutation leading to Sandhoff's disease affects the active site of the enzyme, thus both conformers are absent, and that that leading to Tay-Sachs disease causes instability of the 'A' conformation. In the light of this new claim it might then be worth studying the properties of the heterokaryon hexosaminidase A in rather more detail. It would not, however, seem surprising if the task were unrewarding because despite the wealth of arguments concerning the biochemical and immunological properties of the enzymes one simple yet important set of findings is not readily compatible with this one gene model. This also comes from somatic cell hybrid studies. In human/mouse hybrid cells (in which many of the human chromosomes are lost) the human hexosaminidase A could be lost without B, and its loss has been associated with the loss of a particular human chromosome (Lalley *et al.*, *Proc. natn. Acad. Sci. U.S.A.*, **71**, 1569; 1974).

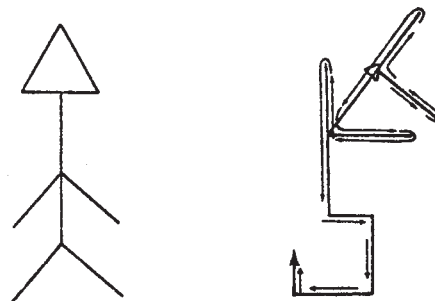
Complementation then of these two mutant lines does not seem to have solved the hexosaminidase puzzle, but it does provide another example of virus fusion in which interesting observations can be made from heterokaryons without the use of a system to select for mononuclear hybrid cells. The success of this experiment should provoke somebody into attempting the same thing with other mutant hexosaminidase lines—which might just turn out to be more informative.

From a Correspondent

Bugs are beautiful

from our Experimental Psychology Correspondent

It might seem that using the computer as a theoretical vehicle in the exploration of the mind constitutes about as reductionist a strategy as one could devise: likening thought to processes involving punched cards and electronic circuits. But to judge from the discussions at the Artificial Intelligence Society of Britain (AISB) Summer Conference held at the University of Sussex from July 8-10, many of the



Left: intended drawing of stick man; right: attempt by program with bugs (arrows represent tracks of pen).

practitioners of artificial intelligence take up on the contrary a very mentalist stance indeed. A couple of speakers who described their work as simulating neural processes, or making computational models of the cortex, attracted energetic comment from other participants.

Although the size of the conference indicated that artificial intelligence is now well established in Britain, the stars of the show were undoubtedly the speakers from the large American centres of AI, and in particular from the Massachusetts Institute of Technology. In that region at least it is neither the potential for stimulating neurones, nor the automation efficiency of the computer that is a source of light on the nature of intelligence at present. The elucidation emanates from an examination of 'bugs'—the programming errors which prevent the actuality of programs from reflecting the aspirations of the programmers.

Typical of this kind of work at MIT is I. P. Goldstein's program which takes a specification of a simple picture, in descriptive terms, including the names of its parts (for example, head, body, legs) and relational statements such as that the legs and body are connected, the arms are below the head and so on. It also has as part of its input a program intended to draw a stick man, which represents an approximate solution, but which has bugs in it (see figure). What the debugging part of Goldstein's program knows is a good deal about the structure of simple programs in which parts (for example, procedures) can be corrected and executed independently of other parts. It also has some problem specific knowledge about how to relate descriptions in the non-procedural descriptive statement of the specification to the primitives of the graphical procedures which can draw pictures. What the whole program does in effect is to edit and rewrite parts of itself in the light of its failures to achieve its specific performance.

This provides a view of the problem solving process in which a coherent strategy, albeit containing misconcep-

tions and shortcomings, is executed. The mistakes so generated are regarded as valuable rather than unfortunate, because they can give the information necessary to improve the program's or the programmer's grasp of the process. This view is, for instance, quite different from the approach to problem solving taken in the General Problem Solver, or in most game playing programs, where absurdly large numbers of microscopic actions are searched through to see if the program can stumble across a sequence of moves that provide a solution. That approach is the domain of the combinatorial explosion: the number of moves to be searched grows astronomically as the program peers rather blindly ahead.

Programs which debug themselves, like Goldstein's, or G. J. Sussman's program called 'Hacker' whose skill improves with practice, or the construction of a programming apprentice which is being designed by C. Hewitt and his colleagues (MIT) to cooperate in the design and testing of a program, represent what looks like a productive approach to problem solving. Mistakes are valuable in that they represent interaction of a program with its environment, the experimental disconfirmations generated by a programmed theory about how to do something. An initial strategy for designing a process will almost certainly fall short in some way, but if the programmer gets about it properly, the nature of the failure can indicate a new and better theory of how that process works or can be designed.

The emphasis on bugs and debugging constitutes another new departure in AI. Bugs provide a further way in which interaction with computational forms extends the understanding of intelligence. The task is not really to simulate the brain, nor to discover that intelligence is so hopelessly complex that we continually find ourselves trying to defuse one combinatorial explosion, after another. It is more a case of providing ourselves with better ways of thinking about thinking: more penetrating and productive metaphors for the mind than Freud's bubbling cauldron of the unconscious, or the self-operating supermarket door of stimulus-response theory. New insights into thought processes may emerge from looking at the nature of bugs and the ways they are made, and from the attempt to formalise the process sufficiently to write programs that debug themselves. It can then be seen if the experience and understanding gained encourages thought not just about program bugs, but how people do learn, or could learn, from their mistakes.

Sahara spreads south

from Peter D. Moore

DURING the past century the Sahara Desert has expanded very considerably, particularly in a southerly direction. As a result the lands along the southern edge of the Sahara, which have long been used for grazing by nomadic tribes, no longer support savanna grasslands but have been engulfed by the desert.

Recent attempts at explaining this expansion have centred around the concept of climatic change; for example, Lamb (*Phil. Trans. R. Soc.*, **A276**, 195; 1974) has reviewed evidence for climatic changes during the past 5,000 years. Although he considers it impossible as yet to construct vegetational maps for early civilised times, there is evidence which suggests that the Sahara has enjoyed higher levels of rainfall. Rock drawings of animals have been found in western and central areas of the Sahara which suggest that game was once available where it no longer exists. Skeletons of elephants have even been found, so some surface water must then have been available. The level of Lake Chad (southern Sahara, 13°N) was more than 30 m above present day levels 5,000 yr ago; also, at that time, the level of the Nile floods was far greater than has regularly been experienced since. In Lamb's opinion, the equatorial rains ranged farther north during their seasonal migration which could have resulted in a regular rainy season to 20°N and erratic rains further north still. As a result one would expect a greater abundance of subsoil water than is now the case.

A consideration of rainfall patterns in the area during the past 70 yr (Winstanley, *Nature* **245**, 190; 1973) has shown that there has been a considerable decrease in rainfall in the southern Sahara since 1960 (from 165 mm to 100 mm in 1970, using 5 yr running means). In Winstanley's view this represents a southerly shift in isohyets of 9 km per year. Since there is a steep spatial gradient of rainfall in this area this could account for the spread of desert conditions.

Back in 1952 a rather different view prevailed. Richards (in, *The Tropical Rain Forest*, Cambridge, 1952) considered that the expansion of savanna grasslands at the expense of rain forest south of the Sahara was the result of an increased use of fire by man in the area. Similarly the spread of the desert has often been attributed to human mismanagement. This view has recently been reiterated by Cloudsley-Thompson (*Envir. Conserv.*, **1**, 5; 1974). He considers that the use of fire by man, which can now be traced back

more than 50,000 yr in East Africa, is a critical factor in the development of savanna and the subsequent spread of desert, but that the additional pressures imposed by overgrazing also deserve attention.

Undoubtedly overgrazing has been an important factor, especially where goats have been involved, and it can result in the production of desert-like conditions even in situations where rainfall is moderate, for example 635 mm in Karamoja, Northern Uganda. In addition to the removal of vegetation cover, the influence of trampling on soil erosion may also be considerable.

It is, however, impossible to ignore Winstanley's data or to regard them as unimportant. The response of vegetation to climatic change in the absence of human-induced factors is essentially slow, as the result of the long generation time of many climax-dominant species and to the influence which plants have on local microclimate because of their canopy structure. Thus vegetation, particularly if it is structurally complex as in the case of forest, possesses an inertia which buffers it against minor climatic fluctuations. One of the most important influences of man on world vegetation has been the simplification of the structure of habitats by felling, burning and grazing, with a resulting modification of microclimatic conditions close to the ground. Soil hydrology and nutrient content are also affected by such processes. It is likely that the high intensity of grazing imposed on the savanna south of the Sahara has rendered the vegetation of the area particularly sensitive to the changes in rainfall pattern. The result has been the spread of the Sahara.

Galactic chemistry

from Virginia Trimble

THE problems of determining the abundances of the chemical elements and accounting for them in terms of a theory of nucleosynthesis and the evolution of our Galaxy involves almost every branch of astronomy, and many related sciences, from objective prism spectroscopy and neutron activation techniques to nuclear shell models and computerised histories of the Galaxy. It is not, perhaps, surprising then that no single, clear picture of nucleosynthesis and evolution emerged from the NATO Advanced Studies Institute on the origin and abundance of the chemical elements, held at the Institute of Astronomy (Cambridge) from July 22 to August 9. If there is any unifying principle at all, it seems to be that, wherever one looks, there is evidence that more than one

process has been at work, and that, therefore, no one mechanism or theory can hope to explain all the observed chemical and isotopic abundances.

Very broadly, one must think in terms of stars more massive than the Sun undergoing nuclear reactions throughout the history of the Galaxy and returning the products of those reactions to the interstellar medium. Successive generations of stars, including the Sun and its planetary system (whose composition is, by definition, normal and the standard with respect to which enhancements or deficiencies elsewhere are measured) will then be formed from the material thus enriched in elements heavier than hydrogen and helium.

Within our Solar System, abundances can be determined for meteorites of various types, the solar photosphere, the solar corona and wind, and particles ejected by solar flares, as well as for the Earth (which is, however, so chemically fractionated as to be almost useless as an indicator of general abundance). The carbonaceous chondrites (which are the type richest in volatile materials and therefore the closest to the primitive solar nebula in composition) have, to first order, compositions and structure which can be understood in terms of temperatures at which various compounds condense from a gaseous to a solid phase, with some later remelting and fractionation of their parent body, according to E. Anders (University of Chicago). But there are additional components, amounting to 1 or 2% of the masses of the meteorites concerned which, from their isotope ratios (in one case a deficiency of ^{20}Ne and ^{21}Ne relative to ^{22}Ne , and in the other an excess of ^{18}O relative to the other isotopes), seem to represent interstellar grain material incorporated into the meteorites without ever having been vaporised and recondensed in the solar nebula, according to R.N. Clayton (University of Chicago). The Earth on the basis of its oxygen isotope ratio may also contain about 1% of this unvaporised component.

High energy particles ejected from solar flares exhibit strong variations in composition with energy below a few MeV per nucleon, but above that converge to abundances which are not significantly different from those in the solar photosphere or corona. This suggests, according to P. B. Price (University of California, Berkeley) that, at sufficiently high energies, the composition of cosmic rays coming from outside the Solar System might also reflect that of the objects which are their sources (supernovae or supernova remnants in most theories). In fact, at those energies which have been studied, the cosmic ray composi-

B and H

from Peter J. Smith

As far as units are concerned, geomagneticians have traditionally had an easy life. Geomagnetic measurements are usually made in media having relative permeability $\mu_r \sim 1$; and in the c.g.s. e.m.u. system the permeability of free space $\mu_0 = 1$. Thus the induction B is related to the field intensity H by $B = \mu_r \mu_0 H = H$ for all practical purposes. As a result of this simple equation geophysicists have seldom had to think very carefully about the physical difference between B and H ; few, if any, have really cared whether their magnetometers have measured B or H ; the gauss, the c.g.s. unit of B , has by usage become totally interchangeable with the oersted, the c.g.s. unit of H ; and as Lowes points out (*Geophys. J.*, **37**, 51; 1974), it is no longer even clear whether the gamma was introduced as 10^{-5} gauss or 10^{-5} oersted.

The recent recommendation by the International Association of Geomagnetism and Aeronomy

tion shows unmistakable signs of spallation (which greatly enhances the amount of Li, Be, and B among other things) as a result of passage through the interstellar medium. After corrections for spallation, the apparent cosmic ray source composition, as determined by M. Shapiro (Naval Research Laboratory) and others, still has a great enhancement of heavy elements in general over hydrogen and helium and of the iron peak elements with respect to C, N and O. There may, in addition, be an excess of U and Th and other nuclei produced in the r process (in which neutrons are captured rapidly by iron peak nuclei and the resulting unstable products then beta decay back to stability). The cause of these anomalies must be apportioned between the processes which produce the nuclei that become cosmic rays and the processes that accelerate the nuclei to relativistic energies. It is not clear how this apportionment should be made.

A recently discovered, low energy component in the cosmic rays has a quite different set of anomalies (O and N enhanced with respect to C, and enhanced He which is pure ^4He). These have been attributed on the one hand to processes in the sources, assumed to be ordinary novae, by D. Clayton (Rice University) and F. Hoyle (University of Manchester) and, on the other hand, to preferential leakage into the heliosphere of atoms with high ionisation potential, by R. Ramaty and

(IAGA) that the geophysical community adopt the SI system has given cause for a little thought, although insofar as the conversions from c.g.s. may be made by simple numerical factors, life will probably go on much as before. Indeed the basic decision about whether to express results in terms of B or H (necessitated by the fact that in SI, $\mu_0 = 1$) was made by IAGA in favour of B in the "interest of achieving... the least possible disruption of existing numerical usage" and in spite of protestations that "matters of physical fact are involved" (Whitworth and Stopes-Roe, *Nature*, **234**, 31; 1971).

In any event, Lowes has now shown that, whatever it may say on the label, real magnetometers have calibrations which depend on the permeability of the medium in which the field is measured. Magnetometers thus actually measure neither B nor H exactly; and so from the instrumental point of view there is no reason for preferentially choosing either for expressing magnetic field data. Not that this will prevent controversy in the future, of course!

others at Goddard Space Flight Center, where the new component was discovered.

The most striking recent progress in measuring abundances has been made by a group at Princeton University as a result of Copernicus observations of ultraviolet interstellar absorption lines. They have obtained a relatively coherent picture, in which the elements that are most depleted from the general interstellar gas onto grains are those that condense at the lowest temperatures. This is reminiscent of the situation in meteorites.

Among the stars, the extraordinary enrichment of the surfaces of certain peculiar A stars in Eu and other rare earths seems, from the work of G. Michaud (University of Montreal), to be attributable to diffusion in the outer layers of stars with initially normal composition. A new class of CH subgiants, discovered by H. Bond (Louisiana State University) has, on the other hand, overabundances of C, Nd, Ce and La, combined with a deficiency of other metals, which seem to reflect mixing to the surface of material which has undergone nuclear reactions in the interior of the stars.

The theoretical models of element production tend to reflect the multiplicity of components in the observations. Several theorists, including I. Iben (University of Illinois) and S. Woosley (California Institute of Technology) have followed the assorted processes (hydrogen and helium burning; hydro-

static and explosive carbon and oxygen burning; explosive silicon burning; and the rapid, slow, and equilibrium capture of neutrons) which should occur in the various layers of normal stars. By a judicious mixing of the products of the various processes (corresponding roughly to the masses in each of the layers), they have succeeded in reproducing the observed abundances in the Solar System over a wide range of atomic numbers while using a rather small number of adjustable parameters.

On a still larger scale, W. D. Arnett (University of Illinois) and R. Talbot (Rice University) and B. Tinsley (University of Texas, Dallas) have formulated models of the evolution of our Galaxy as a whole which can reproduce the variations of total metal abundance with time and position in the galaxy as well as the percentages of matter now present in the form of gas and of stars of various masses and compositions. The models are by no means unique.

Lizards and dune buggies

from our Animal Ecology Correspondent

NOBODY can doubt that increased leisure time available to people living in affluent societies is on the verge of creating immense environmental hazards. As it seems likely that time and money are going to be increasingly plentiful in the future, it is high time that ecologists and politicians got together to formulate useful legislation. This must be tailored to suit the specific ecosystems under pressure and no area, however barren, should be thought to be unworthy of inclusion.

The vast Mojave desert of the southwestern United States is one such barren area that has been used for more than a century for mining and domestic stock grazing. Today it is an important playground for the people of California and, as might be expected, the huge pressure so induced is taking its toll. At a conservative estimate there are 1.2 million motor cycles and 500,000 dune buggies in California most of which are used for scrambling and other off-road pursuits. A most useful pilot study of the impact of both vehicles and sheep on the Mojave has been made by Busack and Bury, and it is to be hoped it does not escape the attention of environmental decision makers (*Biol. Conserv.*, 6, 179-183; 1974).

Dove Springs canyon, 23 km N and 9.5 km W of California City, was chosen as a study site since it is a popular place for buggy racers to display their talents. Three 1 ha plots representing heavy use, moderate use and no use were established and all the

resident lizards in each were collected during a 3-day period. The conditions under which the census was made satisfy the restrictions as to effectiveness laid down by Zippin (*Biometrics*, 12, 163-189; 1956). Some lizards were caught by hand (the rare ones which were subsequently released), some were shot with 0.22 dust shot and others were shot with elastic bands. In the heavily used area, where the vegetation was severely reduced, only two lizards (*Uta stansburiana*) were caught. In the moderately used area 15 lizards were taken adding *Callisaurus draconoides* and *Cnemidophorus tigris* to the list. The biomass of lizards in this area was more than 48 times greater than that in the first area. The unused plot yielded 24 lizards (the three species mentioned plus *Crotaphytus wislizenii*) and had a biomass almost double that of the moderately used area. If lizards can be taken as indicator species of general ecological complexity—and being insectivorous this seems not unreasonable—there seems to be a case for speedy legislation to restrict dune buggy racing only to certain areas.

Grazing has different effects on lizards. Previous studies of desert vegetation have indicated that grazing does not greatly affect the diversity of plant species (Blydenstein, Hungerford, Day and Humphrey, *Ecology*, 38, 522-526; 1957; Gardner, *Ecology*, 31, 44-50; 1950). Protection from grazing serves to increase biomass without seriously affecting ecological complexity. In Gardner's study 30 years' protection from grazing brought about a 110% increase in biomass. The ungrazed study plot used by Busack and Bury yielded 36 lizards with a total biomass of 690 g ha⁻¹; of this 6 *C. draconoides* comprised less than 10% of the total biomass. The plot which had been heavily grazed by sheep in the previous year revealed 17 lizards with a biomass of 185 g ha⁻¹. Interestingly, of this 17, 11 were *C. draconoides* which comprised almost 70% of the total biomass. *Phrynosoma platyrhinos* and *C. wislizenii* were absent from the grazed plot. The likely interpretation of these observations is that the vegetational changes associated with grazing reduces to below an acceptable exploitable level the population of insects required by these species. *C. draconoides* is an agile lizard that favours open areas with scant plant growth and small rocks. The effect of grazing is to produce these conditions.

Both motor cycling and buggy racing have an important influence on community structure which could result in irreparable ecological damage. Strict control over who does what where in the Mojave will be required if the whole place is not to become unfit for lizards and thus, in the long term if not in the short, unfit for any kind of human use.

Lattice defects in Freiberg

from John Walker

THE latest in the series of international conferences on lattice defects in semiconductors, held at Freiberg from July 22-25, was opened by G. D. Watkins (General Electric, Schenectady) with his traditional review of spin resonance studies in silicon. The self-interstitial has still not been isolated, but seems to be produced in a positive-charge state during irradiation. It is then trapped by and changes place with the negatively-charged acceptors in p-type material. This trapping does not occur in n-type material, so the damage rate is lower, though boron counterdoping increases it. The importance of oxygen in radiation damage was emphasised by the identification of the Si-G3 and Si-G4 centres as vacancies perturbed by oxygen atoms. Both defects anneal to give the well-known A-centre (an oxygen-vacancy pair).

R. P. Messmer (General Electric, Schenectady), in his review of the theory of point defects in semiconductors, described the X α scattered wave theory which has been used to illuminate the controversy between many-electron and one-electron treatments of the diamond vacancy. Apparently the electrons are not sharply localised at the defect, but spill out into the surrounding lattice. Hence electron-electron interaction is reduced and the one-electron calculations are adequate. This is consistent with experimental results on silicon, but the data are not available for diamond.

In the diamond session, L. A. Vermeulin and colleagues (Universities of Reading and the Witwatersrand) reported the observation of sharp photoconductivity peaks in irradiated diamond crystals, which suggests the existence of bound states within the conduction or valence bands, J. W. Van der Sande (University of the Witwatersrand) has used the low temperature thermal conductivity of irradiated diamond to demonstrate the presence of interstitial clusters 200 Å in diameter; these indicate that defects are mobile below room temperature.

Phosphorus ions, when ion-implanted into silicon in a random direction, penetrate further than expected. Explanations which have been suggested are diffusion and the scattering of ions into channelling directions. P. Blood and colleagues (Mullard Research Laboratories, Redhill and AERE Harwell), in a very elegant experiment, prepared a thin silicon crystal and implanted radioactive phosphorus ions into it. Diffusing ions would necessarily remain in the crystal,

whereas channelled ones could emerge from the back face and be trapped in a second, adjacent crystal. Radioactive ions were found in the second crystal, so channelling was indeed occurring.

D. V. Lang and L. C. Kimerling (Bell Laboratories) introduced a powerful new tool for the study of defect states within the forbidden gap. Deep level transient spectroscopy can detect the activation energy for thermal emission from a defect level to the band edge, the defect concentrations, their spatial profile and the electron and hole capture cross sections. All these parameters are of obvious interest to device manufacturers as well as academic physicists. A voltage pulse fills with charge carriers the defects within the depletion region of a p-n junction. The trapped carriers are then released and cause a capacitance transient whose magnitude and time constant, combined with the device temperature and the width of the depletion region, indicate the above parameters. The technique is also relatively cheap and simple to operate.

Genetical hazards of pollutants

from A. D. Bradshaw

HUMAN beings are rather ambivalent to problems of pollution. On the one hand people can get very worried by them, and yet on the other hand they all too easily dismiss them. It was for this reason that the Genetical Society held a symposium on the subject on July 10–12 during their summer meeting at Lancaster. Eight invited speakers provided an assessment of the current state of knowledge about the genetical effects of pollution.

These effects fall into two distinctive parts—selection and mutation. It was clear that a great deal is now known about the selective effects of pollutants. Indeed, they provide some of the most elegant examples of evolution in action as was shown in the accounts of industrial melanism in moths by L. M. Cook (University of Manchester) and J. A. Bishop (University of Liverpool) and of heavy metal tolerance in plants by A. D. Bradshaw (University of Liverpool). These two examples are remarkably similar in the way they demonstrate the powerful effects of selection in the formation of highly localised adapted populations, particularly in organisms with limited powers of dispersal. In both cases the observed clinal patterns can be correlated with observed values for gene flow and selection.

Although the rapidity of the evolution of insecticide resistance is now well known it is doubtful if many

people realise the remarkable rate of appearance over just a few years of resistance to first one new insecticide and then another. This problem was outlined by R. J. Wood (University of Manchester) who showed how much the evolution of insecticide resistance is ruining insect control. The evolution, however, is determined by the availability of appropriate variation as well as by selection, for only some, and not all, species have evolved resistance.

It is easy to appreciate that evolution of resistance to insecticides or heavy metals, being so much a matter of survival or extinction, will be very rapid: one or two years seem to be sufficient for substantial evolutionary change. It was therefore interesting to hear from R. W. Snaydon (University of Reading) that even the quiet fields of Rothamsted Experimental Station can provide examples of equally far reaching and rapid evolution in the grass *Anthoxanthum odoratum*, in response to the different liming and fertiliser treatments of the Park Grass Experiment. Here indeed is an example which shows the very pervasive effects of evolution on all characters of an organism, and the fact that an ordinary meadow can readily generate coefficients of selection as high as 0.5 or more.

By contrast, contributions on the mutational aspects of pollution did not paint quite such a clear picture, not because of lack of importance of the subject or of lack of work, but because of the inherent difficulties of mutation research. C. E. Purdom (Fisheries Laboratory, Lowestoft) described the history of research on the effects of ionising radiation, from the early days when only the linear relationship between mutation and dose was known to the present day when it is realised that the relation is not so simple and that different species including man can show very different patterns of sensitivity.

This theme was taken up by M. F. Lyon (MRC Radiobiology Unit, Harwell) for the particular case of the effects of radiation on mammals. It now seems that repeated small doses give a lower mutation rate than the same total dose given in a single exposure. Since environmental radiation is received almost entirely in small doses or at low rates it may therefore be less hazardous than was previously thought. At the same time the yield of mutation in mice, and guinea-pigs and hamsters, suggests that mutation yield decreases with time after irradiation. All this is comforting to human beings, but there is still, for obvious reasons, no direct evidence.

The last but not least effect of environmental mutagens, particularly chemicals, is to cause chromosome change. There is enormous pressure to

produce effective means for testing the effects of new chemicals on human tissue. M. L. O'Riordan and H. J. Evans (Western General Hospital, Edinburgh) showed the value of peripheral blood lymphocytes for this purpose. But the problem is to know how far it is possible to extrapolate from such data to effects on germ cells. Certainly germ cells and embryos in human beings have their own particular properties. E. Alberman (Guys Hospital, London) very effectively brought the symposium to a close with an analysis of the causes of spontaneous abortion in human beings. It is very clear from the relation between foetal death and the presence of chromosomal abnormalities in the foetus, and between mean ovarian X-ray dose and foetal death, that abortion is a process by which human beings get rid of much newly acquired genetic abnormality.

Pacific motions

from Peter J. Smith

DEEP sea sediment cores—unlike rocks from continents, oceanic islands and seamounts—have not generally been used to reconstruct lithospheric plate motions. There are several reasons for this: azimuthal orientations of sediment cores have not usually been available, thereby making it impossible to obtain the complete palaeomagnetic information; cores have often been short, thereby limiting data in time; and there are the usual problems of dating and determining sedimentation rates, especially beyond the well defined continental polarity-time scale. Perhaps even more importantly, the sediments easier to recover are obviously the younger ones within whose span plate motions have been small. Ocean cores have therefore been most useful in polarity studies, in elucidating time-stratigraphic relationships and in determining geomagnetic field properties, although some years ago Sclater and Cox (*Nature*, **226**, 934; 1970) did show that cores can be used to trace palaeolatitude variations given adequate time coverage.

Another attempt to determine plate motions from sediments has now been made by Hammond *et al.* (*Earth planet. Sci. Lett.*, **22**, 22; 1974) using 7 cores from the central equatorial Pacific. Taken together the cores spanned completely the period 0.1–21 million years (Myr) ago, a range determined partly from magnetic and partly from biological data. For the younger sediments, dating was based primarily on comparison with the continental polarity-time scale. Sediments in the range 5–10 Myr were dated by compar-

ing the relative lengths of polarity epochs in the cores with those of dated ocean floor anomalies. For the period beyond 10 Myr ago, dates were based largely on the correlation of observed radiolarian zones with the tropical radiolarian zoning previously established.

Such dating is easier to describe than to carry out; but within the limitations of the various methods, Hammond and his colleagues go on to plot palaeolatitude (from the measured palaeomagnetic inclinations) as a function of age throughout each core on the assumption that during the relevant period the geomagnetic field has been axially dipolar. In each case, the variation of palaeolatitude with time indicates northward motion. The overall average rate of movement, based on linear least-squares regression analyses of the latitude-age data, is 8.4 cm yr^{-1} during the past 21 Myr. The average, however, conceals a significant mid-period change in motion, for about 12 Myr ago the rate of northward movement seems to have slowed from about 11 to 6 cm yr^{-1} . This could represent a genuine decrease in the absolute rate of motion, a change in direction (which could reduce the northward component of motion without necessarily reducing the actual rate), or both.

Numbers apart, the important general point demonstrated by Hammond *et al.* is that the method of reconstructing plate motions using deep sea cores can be made to work as long as the cores cover a sufficiently long interval of time. They also note that in view of the limited number of cores studied and the difficulties in dating them accurately, their own particular results must be regarded as tentative. Nevertheless, they draw attention to the fact that their overall drift rate of 8.4 cm yr^{-1} is in excellent agreement with the rate of about 8 cm yr^{-1} obtained by Grommé and Vine (*Earth planet. Sci. Lett.*, **17**, 159; 1972) from a palaeomagnetic study of Miocene basalts from Midway Atoll.

On the other hand, Hammond and his coworkers do not mention that Winterer (*Bull. Am. Ass. Petrol. Geol.*, **56**, 63; 1972) concluded (on the basis of eastern Pacific data) that the Pacific plate has been moving northwards at a rate of only 3 cm yr^{-1} for the past 30 Myr, nor that Heezen *et al.* used western Pacific data to obtain an even lower rate of 2 cm yr^{-1} . But Forristall (*Geophys. Res. Lett.*, **1**, 131; 1974) does mention this previous work, and uses it to expand some ideas of his own concerning Pacific motions.

Forristall's ultimate conclusion is that the asthenosphere beneath the central Pacific is about twice as thick as the lithosphere—a result which will cause little surprise insofar as it is in

general agreement with majority views favouring shallow convection. But the assumptions and data upon which the conclusion is based are much more contentious. For example, many will surely quarrel not only with Forristall's view that the concept of hot spots fixed with respect to each other has been conclusively rejected but also with his more fundamental assertion that "it is hard to escape the idea that linear chains of volcanic features . . . are the expression of isolated subsurface hot spots".

Be that as it may, the more important point in the present context is that in his calculations Forristall uses the northward drift rates proposed by Winterer and Heezen *et al.*, apparently rejecting the higher rate obtained by Grommé and Vine. It thus becomes clear that not only are different workers obtaining quite different figures for recent Pacific plate motion, the variety of values available provides a basis for wider conclusions. The reasons for the discrepancies in drift rate are not difficult to imagine; but until they are sorted out the confusion is likely to increase.

Observing the millenium

by John Gribbin

ON the face of things, the achievement of 1,000 issues of a journal may seem unremarkable by *Nature's* standards—this journal is now well into our sixth 'millenium' of continuous publication. But when the journal in question only appears bimonthly at best, and has recently suffered more than most from the rigours of the British three-day week, there is perhaps some excuse for dropping the mask of scientific sobriety for one celebratory issue.

The journal to which I refer is *The Observatory*, which is the house journal of the Royal Astronomical Society. The sense of humour needed to run such a journal for a body of scientists as free-ranging in their ideas as astronomers is often apparent in ordinary issues of the journal, where sober scientific papers rub cheek by jowl with letters which are sometimes decidedly peculiar (but must presumably be published if they come from Fellows of the RAS?) and with verbatim reports of meetings which are so deadpan that they have on occasion been known to reduce an astronomical coffee break to something approaching hysteria.

To anybody who has been present at the meeting being reported in any particular issue of *The Observatory*, the favoured game is to spot who has taken the opportunity (offered in the

best traditions of scientific accuracy) to change what they actually said at the meeting to what they would have said if they had either remembered, or had time, or read the papers they should have read in advance. Together with the frequent interjections from "A Fellow" (anybody present who was not recognised by the RAS scribes), this makes the meeting reports just about beyond improvement—so it is a pity that the editors of the journal thought it necessary to start their celebratory 1,000th issue with a spoof meeting report.

The spoof scientific papers are more successful, although most of them hinge upon 'in' jokes which will mean little to non-astronomers (but then, who else will be reading them?). But a couple of contributions deserve at least passing notice from the wider world of science. These are concerned with two newly discovered manifestations of the 11-year cycle which seems to be linked to many terrestrial phenomena, and may be triggered by solar activity. Research into this subject is still seen as a contentious issue in some quarters, and one of the contributors hides behind the pseudonym "Disgusted, Tunbridge Wells" (presumably A. Fellow?). What Disgusted has discovered, among other things, is that an analysis of the number of pages in each issue of *The Observatory* shows "a suggestion of the eleven-year cycle which occurs in most astronomical data". But the second communication is even more remarkable.

According to Mathews (*Observatory*, **94**, no. 1,000, 13P; 1974) there is a correlation between sunspot activity and the political "colour" of British governments. Labour governments, it seems, tend to be returned at times of sunspot minimum, and Conservative governments at times of sunspot maximum. Best of all, in a prediction made before the most recent British election but only now appearing in print, Mathews said "regions of political instability may well be triggered in the next few months, resulting in the election of a Labour Government", and also predicted "the continuation of the Liberal revival". Those predictions, of course, may well be considered still relevant today, and if Mr Harold Wilson is a reader of *The Observatory*, there might well be another election in Britain in the very near future.

This area of sunspot research is clearly an important field of astronomy today (see *Nature* **246**, 453; 1973). If there are still doubters who are unconvinced of the reality of the solar-terrestrial links let them ponder on a quote from *The Observatory* of 1877 (**1**, 370): "M. Tempel supposes that the spiral shapes (of Nebulae) are only creatures of fantasy".

Primate social organisation and ecology

T. H. Clutton-Brock

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Attempts to relate interspecific differences in social organisation among primates to gross differences in habitat or diet type have been largely unsuccessful. This is probably partly because distantly related species have adapted to similar ecological situations in different ways and partly because much finer ecological differences are important.

THE accumulation of primate field studies in the course of the last two decades has emphasised the extent to which social organisation varies between species. Marked inter-population differences have been observed in a number of cases¹, but most species possess characteristic modal patterns of social organisation. Members of some species are solitary² for much of their time, while others live in family groups^{3,4} and others in semi-stable groups of fifty or more animals^{5,6}. Groups may include approximately equal numbers of males and females⁷ or, as is more usual, there may be a preponderance of females^{8,9}. In a number of species, groups regularly split up into parties which forage separately^{10,11} while in other all members of the group stay together^{6,8}. The average size of the area used by groups differs from <0.01 to >50 km² (ref. 12).

At present, little is known about the adaptive significance of these differences. Several reviews have attempted to relate them to gross differences in diet or habitat type¹²⁻¹⁸. The first and perhaps the most influential of these was produced by Crook and Gartlan¹³ in 1966. Primate species were divided into five "grades" according to the nature of their diets, the kind of habitat they occupied and the pattern of social organisation which they displayed. The paper emphasises the similarity of social organisation in species sharing the same diet and habitat type and is widely quoted as evidence that social organisation and ecology are closely related. But when it is viewed in the light of current knowledge, differences between groupings are less impressive than differences within them. For example, *Saimiri sciureus*, *Colobus* spp., and *Gorilla* are all placed in the same grade, despite the gross differences in diet, foraging behaviour and social organisation which exist between them¹⁹⁻²⁶. In addition, the paper illustrates a fundamental problem in classifying primate social systems. Since different aspects of social organisation are not well correlated across species, categories defined by a single criterion will include social systems which differ widely in other ways.

Jolly¹² avoids this problem by considering different aspects of social organisation separately. She groups species into six ecological divisions ("nocturnal", "arboreal leaf-eaters", "arboreal omnivores", "semi-terrestrial leaf-eaters", "semi-terrestrial omnivores" and "arid country species"), and compares troop size, range and territory size, population density, inter-group behaviour and day-range size separately between ecological groups. Again, differences within most groupings are more striking than differences between them. For example, between species allocated the "arboreal leaf-eaters" category, average troop size differs from <10 to >50 and average range size from <0.02 to >1 km², while other categories show even more variability.

To criticise these reviews in the light of current knowledge is not to discount their value. The paper by Crook and

Gartlan in particular has stimulated much interest and a considerable body of research. There are, however, theoretical reasons why social organisation should not be expected to be closely correlated with gross ecological variation.

Phylogeny, ecology and adaptation

One probable reason for the absence of close correlation is that different species tend to react to similar environmental pressures in different ways. When a novel adaptation evolves, its form will be partly determined by the various environmental factors through which selection is operating and partly by the species' phylogenetic inheritance^{27,28}. Consequently, distantly related species are likely to evolve different traits with similar functions. For example, anatomical specialisations permitting the digestion of foliage have evolved in several primate families. In some this has been achieved by the development of a large caecum (for example, *Alouatta*, *Indri*)²⁹, in others by chambered stomachs with bacterial symbionts (for example, *Colobus*, *Presbytis*)³⁰. Similarly, many interspecific differences in social organisation may well prove to represent different methods of overcoming the same ecological problems.

Another reason is that the wrong kind of ecological variation has been assumed to be important. Within habitat categories as broad as "forest", "woodland" and "savanna", and dietetic categories such as "insectivore", "frugivore", "folivore" and "omnivore" there is room for vast ecological differences. Food supplies may be static or mobile, sparse or dense, heavily clumped or evenly dispersed, reasonably

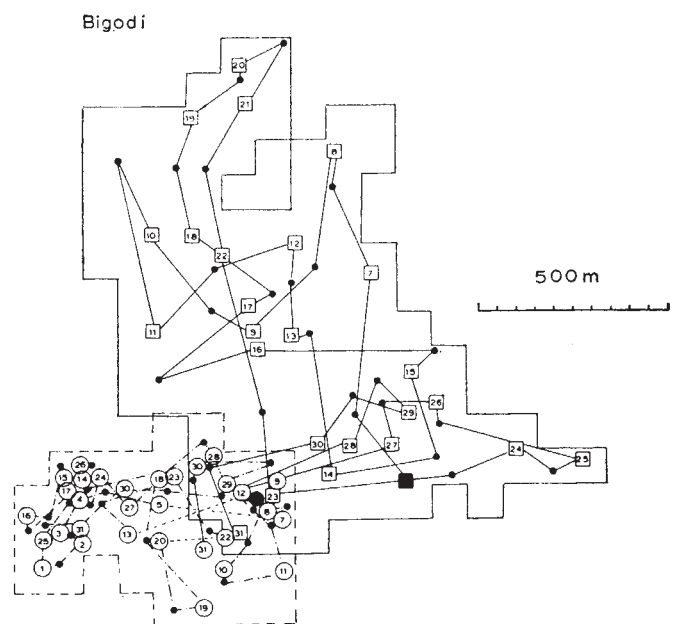


Fig. 1 Movements of a troop of red colobus (squares: September 6 to 31, 1970) and one of black and white colobus (circles: August 29 to September 31, 1970) about their ranges in Kibale Forest Reserve, Uganda. Squares and circles shows the troops' night-resting positions, the figures inside them the dates. Points show the positions of the troops at 12 noon on each day.



Fig. 2 A male black and white colobus watches an intruder.

stable throughout the year or extremely variable¹⁷. Intensity of predation may vary in the same ways. If ecological differences at this level affect social organisation (and, by analogy, the plentiful literature on birds suggests that this will be found to be the case²¹) one should expect social organisation to vary widely within habitat and diet types.

A recent study of two species of colobus monkeys in East Africa³² provides an example of the level at which differences in social organisation may be related to ecology. Both the red colobus (*Colobus badius*) and the black and white colobus (*C. guereza*) are widely distributed across tropical Africa³³. The two species are sympatric throughout much of their range, though the black and white colobus is found both in wet and dry forest while the red colobus is less commonly found in dry forest^{25,34}. Although both species are largely folivorous, arboreal and forest-dwelling,³⁵ their characteristic patterns of social organisation differ widely. Red colobus live in large, multi-male troops of 40 or more animals which occupy extensive ranges of around 1 km² in size (see Fig. 1). Their different calls intergrade³⁶. Oestrous females show pronounced swelling of the perineal region. Infants have a black natal coat and are apparently handled only by the mother during the first months of life. In contrast, black and white colobus live in small troops of five to 10 animals which often contain only one adult male²⁴⁻²⁶. The troops occupy defended territories usually <0.2 km² in size and inter-troop relationships are normally hostile²⁴. Males give a booming roar which may serve to space groups³⁷ while intragroup vocalisations intergrade as in the red colobus. Females show no obvious oestrous swellings. Infants have a white natal coat and are handled by females other than their mothers from the day of birth³⁸.

Between August, 1969, and June, 1970, I observed one troop of red colobus in the Gombe National Park for 9

months³². Afterwards, for approximately a month each, I watched one troop of red colobus and one of black and white colobus in each of two areas in Kibale Forest, Uganda. I measured the amount of time which the animals spent feeding on different foods by recording, at quarter-hourly intervals, the foods that all visible animals were eating³⁹. In each area, I also measured the relative abundance of the different tree species.

The feeding behaviour of all three troops of red colobus was very similar. The animals ate the flowers, fruit, shoots and leaves of a variety of tree species. They were extremely selective in their choice of food, regularly choosing certain parts of particular tree species. Most tree species were not evenly distributed through the forest, with the result that the animals fed on different foods in different parts of their ranges. In addition, the availability of food on most species varied seasonally and this was reflected in seasonal changes in the animals' diet. When shoots, flowers and fruit were less abundant, the animals fed to a greater extent on mature leaves, though in no month did they spend more than 60% of their feeding time eating mature leaves. In all months of the year at Gombe, and in both study areas at Kibale, the animals fed on a wide variety of food species. To measure the variability of their diet, in each month I ranked the animals' foods on the amount of time spent feeding on them. At Gombe the proportion of time spent feeding on the top-ranking food species varied from 13-42% between months, that on the second ranking species from 11-21%, while the amount of time spent feeding on the top five varied from 51-80%. Figures for the two Uganda troops were similar. In one study area the red colobus spent 11% of their time feeding on the top-ranking food species, 10% on the second and 46% on the top five. In the other they spent 16% of their time on the top species, 14% on the second and 50% on the top five.

The diet of the black and white colobus troops differed in two important ways from that of the red colobus. Both troops fed almost exclusively on two tree species (*Celtis durandii* Engl. and *Markhamia platycalyx* (Bak.) Sprague). In one area *Celtis durandii* accounted for 71% of all feeding records and *Markhamia platycalyx* for 19%. In the other, *Celtis durandii* accounted for 88% and *Markhamia*



Fig. 3 A female red colobus leaps from one tree to another, her infant clinging to her chest.

platycalyx for 5%. These species were utilised to a lesser extent by the red colobus troops in both areas and the difference in feeding behaviour was not a product of reduction in the availability of alternative foods to the black and white colobus³⁹. Second, during the first weeks of the Uganda study, when few *Celtis* trees had yet come into flower or shoot, the black and white colobus fed largely on the mature leaves of *Celtis*. At the same time, the red colobus were feeding on the shoots, flowers and fruit of a variety of other food species, but were not observed to feed on mature *Celtis* leaves. Subsequently, many of the *Celtis* trees came into flower and shoot, and the black and white colobus switched to feeding on these parts.

These differences in feeding behaviour may be closely related to the differences in distribution and social organisation between the two colobus species. Black and white colobus may be able to exist on a diet of mature leaves when only these are available. This would allow the species to colonise areas of dry forest where strongly seasonal rainfall produces a high degree of production synchrony across different tree species (so that shoots, flowers and fruit are only available at certain times of year). Second, it would allow them to exist on the products of a small number of tree species throughout the year, since these would provide acceptable food in all seasons. Consequently, a small area of forest would be able to support animals throughout the year. In this situation, small troop size might permit the animals to minimise range size and thus to increase their ability to defend their food supply efficiently^{17,40}. It might also allow them to minimise the distance which they would have to travel each day to collect food (both because the total demands of the troop would be less and because they would be able to utilise food sources too small for larger troops).

In contrast, red colobus may need to maintain a high proportion of shoots, flowers and fruit in their diet throughout the year. Consequently, the species may be limited to wetter forests where some tree species carry these parts in all seasons. Since different tree species will carry acceptable foods at different times, the animals would need to maintain access to a wide variety of food species. As the distributions of most tree species are heavily clumped, a large sized range would be necessary in order to provide sufficient supplies of acceptable food in all months of the year. A square kilometre of forest can support a considerable population of red colobus. Theoretically, the animals could either aggregate into a single group or split up into several small groups with extensively overlapping ranges. In the red colobus, minimisation of group size may be less advantageous because the range size necessary to support even a small group throughout the year would be too large to be efficiently defended. Aggregation may have a number of advantages: the presence of other feeding animals in the immediate area may show individuals where to find food⁴¹, while the troop may provide a reservoir of knowledge about the distribution of food and of predators in the past⁴². It also may allow the animals to develop a pattern of regular use of the different parts of the range which would maintain leaf growth at an acceptable stage and maximise the reaction of the tree species to being cropped⁴³. Finally, large troop size may enhance the animals' ability to detect and defend themselves against predators. Certainly chimpanzees at Gombe were regular predators of red colobus (R. Wrangham, personal communication) and several instances of cooperative defence by the colobus were observed.

The origins of several other differences between the two species are probably linked with these differences in social organisation. The contrasting pelage of the black and white colobus and the males' roars may help to demarcate troops' territories (J. F. Oates, quoted in ref. 44). Differences between the two species' reactions to intruders are well



Fig. 4 Female red colobus feeding on flowers of *Combretum molle*.

adapted to the difference in group size. When disturbed, black and white colobus tend to move to thick cover and remain silent while red colobus escape noisily and males may attack potential predators, including chimpanzees. Other differences, such as the absence of obvious sexual swellings in the black and white colobus and of infant-swapping in the red are less easily explained.

This reconstruction is clearly speculative. Few colobus troops were observed and, except at Gombe, they were watched only at one time of year. Current studies of the two species by T. T. Struhsaker and J. F. Oates may throw further light on the problem. But large group and range size is associated with clumped, unstable food supplies in several other animal groups. Studies of *Presbytis entellus* and *P. senex* in Ceylon show that *P. entellus*, which lives in larger troops in larger ranges than *P. senex* also feeds more on fruit and less on mature foliage and utilises a wider range of foods. Among East African ungulates, most of the plains-dwelling, grazing species, whose food supplies tend to be clumped and relatively unpredictable, aggregate in large herds which range widely (for example, most of the *Alcelaphinae*). In contrast, most browsing species, whose food supplies tend to be more uniformly scattered and more stable, occupy small ranges and live either in small groups, in pairs or alone (for example, dik-diks, duiker and most of the *Neotraginae*)⁴⁶. With some exceptions, the same association between diet and social organisation is found among European and Asiatic deer. Finally, a high proportion of graminivorous bird species feed in flocks, whereas relatively few insectivorous species do so³¹. The diet of the former is commonly assumed to be more heavily clumped than that of the latter⁴⁷. Within the insectivores, the aerial feeders (for example, swallows, martins and swifts) whose food supplies tend to be more clumped and less predictable, almost all feed in flocks³¹.

The colobus study provides one example of the kind of ecological differences which may be associated with interspecific differences in social organisation. In other primate species, different factors are likely to be important. But if many of them are as subtle as those probably involved in

this case, considerable variation is to be expected within gross diet and habitat categories. This is not to say that high level ecological variation has no effect on social organisation. Indeed, the examples quoted above suggest that in some animal groups there is a relatively close correlation between some aspects of social organisation and certain types of diet. Whether associations at this level can be identified will depend both on the extent of variation within ecological categories and on the number of species which can be compared. Lack³¹, working with a vast array of bird species, was able to demonstrate correlations between social organisation and gross dietetic variation despite the presence of considerable variation within ecological categories. In primates, however, this approach is less likely to be useful both because there are relatively few species and because there is evidently wide variation within gross ecological groupings.

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- ¹ Jay, P. C., in *Primates: Studies in Adaptation and Variability* (edit. by Jay, P. C.), (Holt, Rinehart and Winston, New York, 1968).
- ² Charles-Dominique, P., and Martin, R. D., *Advances in Ethology*, **9** (Paul Parey, Berlin, 1972).
- ³ Ellefson, J. O., in *Primates: Studies in Adaptation and Variability* (edit. by Jay, P. C.), (Holt, Rinehart and Winston, New York, 1968).
- ⁴ Mason, W. A., in *Primates: Studies in Adaptation and Variability* (edit. by Jay, P. C.), (Holt, Rinehart and Winston, New York, 1968).
- ⁵ Gautier-Hion, A., *Folia primat.*, **12**, 116–141 (1970).
- ⁶ Altmann, S. A., and Altmann, J., *Baboon Ecology* (University of Chicago Press, Chicago, 1970).
- ⁷ Jolly, A., *Lemur Behaviour* (University of Chicago Press, Chicago, 1966).
- ⁸ Poirier, F. E., *Folia primat.*, **10**, 20–47 (1969).
- ⁹ Hall, K. R. L., *J. Zool.*, **148**, 15–87 (1965).

- ¹⁰ Eisenberg, J. F., and Kuehn, R. E., *Smithsonian Misc. Coll.*, **151**, 1–63 (1966).
- ¹¹ Aldrich-Blake, F. P. G., Thesis, University of Bristol (1970).
- ¹² Jolly, A., *The Evolution of Primate Behaviour* (Macmillan, New York, 1972).
- ¹³ Crook, J. H., and Gartlan, J. S., *Nature*, **210**, 1200–1203 (1966).
- ¹⁴ Crook, J. H., in *Social Behaviour in Birds and Mammals* (edit. by Crook, J. H.), (Academic Press, London, 1970).
- ¹⁵ Crook, J. H., *Anim. Behav.*, **18**, 197–209 (1970).
- ¹⁶ Denham, W. W., *Am. Anthropol.*, **73**, 77–95 (1971).
- ¹⁷ Crook, J. H., in *Sexual Selection and the Descent of Man* (edit. by Campbell, B. G.), (Aldine, Chicago, 1972).
- ¹⁸ Eisenberg, J. F., Muckenkirn, N. A., and Rudran, R., *Science*, **176**, 863–874 (1972).
- ¹⁹ Schaller, G. B., *The Mountain Gorilla* (Chicago University Press, Chicago, 1963).
- ²⁰ Thorington, R. W., jun., in *Progress in Primatology* (edit. by Starck, D., Schneider, R., and Kuhn, H.-J.), (Fischer-Verlag, Stuttgart, 1967).
- ²¹ Baldwin, J. D., *Folia primat.*, **9**, 281–314 (1968).
- ²² Baldwin, J. D., *Folia primat.*, **11**, 35–79 (1969).
- ²³ Baldwin, J. D., and Baldwin, J., *Folia primat.*, **18**, 161–184 (1972).
- ²⁴ Marler, P., *Science*, **163**, 93–95 (1969).
- ²⁵ Kingston, T. J., *E. Afr. Wildl. J.*, **9**, 172–175 (1971).
- ²⁶ Schenkel, R., and Schenkel-Hulliger, L., in *Progress in Primatology* (edit. by Starck, D., Schneider, R., and Kuhn, H.-J.), (Fischer-Verlag, Stuttgart, 1967).
- ²⁷ Chalmers, N. R., in *Comparative Ecology and Behaviour of Primates* (edit. by Michael, R. P., and Crook, J. H.), (Academic Press, London, 1973).
- ²⁸ Struhsaker, T. T., *Folia primat.*, **11**, 80–118 (1969).
- ²⁹ Hladik, C. M., *Mammalia*, **31**, 120–147 (1967).
- ³⁰ Hollihn, von K. W., *Z. Säugetierk.*, **36**, 65–95 (1969).
- ³¹ Lack, D., *Ecological Adaptations for Breeding in Birds* (Methuen, London, 1968).
- ³² Clutton-Brock, T. H., Thesis, University of Cambridge (1972).
- ³³ Rahm, U. H., in *Old World Monkeys* (edit. by Napier, J. R., and Napier, P. H.), (Academic Press, New York, 1970).
- ³⁴ Kingdon, J., *East African Mammals*, **1** (Academic Press, London, 1971).
- ³⁵ Clutton-Brock, T. H., *Folia primat.*, **19**, 368 (1971).
- ³⁶ Marler, P., *Folia primat.*, **13**, 81–91 (1970).
- ³⁷ Marler, P., *Behaviour*, **42**, 175–197 (1972).
- ³⁸ Wooldridge, F. L., *Anim. Behav.*, **19**, 481–485 (1971).
- ³⁹ Clutton-Brock, T. H., *Folia primat.* (in the press).
- ⁴⁰ Goss-Custard, J. D., Dunbar, R. I. M., and Aldrich-Blake, F. P. G., *Folia primat.*, **17**, 1–19 (1972).
- ⁴¹ Krebs, J. R., MacRoberts, M. H., and Cullen, J. M., *Ibis*, **114**, 507–530 (1972).
- ⁴² Kummer, H., *Primate Societies: Group Techniques of Ecological Adaptation* (Aldine, Chicago, 1971).
- ⁴³ Vesey-Fitzgerald, D. R., *E. Afr. Wildl. J.*, **7**, 131–145 (1969).
- ⁴⁴ Marler, P., *Behaviour*, **42**, 175–197 (1972).
- ⁴⁵ Hladik, C. M., Hladik, A., *La Terre et la Vie*, **2**, 149–215 (1972).
- ⁴⁶ Jarman, P. J., *Behaviour* (in the press).
- ⁴⁷ Emlen, J. M., *Ecology: An Evolutionary Approach* (Addison, Welsley, Mass, 1973).

Tectonic segmentation of the Andes: implications for magmatism and metallogeny

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The Andean orogen consists of a series of tectonic segments separated by transverse boundaries that reflect discontinuities on the underlying subduction zone. The characteristics of belts of magmatic rocks and the type, age and size of ore deposits in a series of longitudinal belts may change at tectonic boundaries.

THE central Andes provide a classic example of a volcano-plutonic orogen developed along a convergent plate margin¹. The longitudinal continuity and transverse variability of characteristic volcano-plutonic orogens are commonly emphasised². Although I accept that longitudinal continuity is dominant in such orogens, I stress here the potential significance of a fundamental, longitudinal segmentation of the central Andes and, by analogy, of other comparable orogenic belts above subduction zones.

In Chile a series of longitudinal, physiographic provinces (the Norte Grande, Norte Chico, Central Chile, and Southern Chile) has been recognised for many years. Some of the boundaries between the physiographic provinces must have a seismic significance because they coincide with positions at which the level of seismicity changes^{5,6}. The Norte Grande and Central Chile, with longitudinal, fault-bounded valleys, bordered to the east by lines of recent volcanoes^{3,4,7}, contrast with the Norte Chico from which these features are absent, thereby demonstrating that the physiographic provinces also exert a control over tectonism and calc-alkaline magmatism.

Similar, possibly more important, transverse boundaries which coincide with major features in the east-central Pacific, effectively define the northern and southern limits of the central Andes and mark a single, transverse division⁸.

A study of intermediate and deep focus earthquakes in the Japanese arcs⁹ revealed a series of offsets and changes in strike in the deep seismic zone, which is thus effectively divided into a number of 100–300 km long segments. These discontinuities can be correlated with surface geological features, such as: offsets or changes in the strike of the trench axis or of belts of volcanoes; and lines of volcanoes, prominent structures or topographic changes transverse to the arc⁹. It is proposed here that the well known longitudinal subdivisions of Chile, and the boundaries emphasised by Gansser⁸, are comparable to the transverse geological features in Japan and are probably also coincident with discontinuities on the underlying deep seismic zone.

Using the criteria of Carr *et al.*⁹, together with additional geological evidence, I have subdivided the central Andes into tectonic provinces (Fig. 1).

Definition of tectonic segments

Boundary 1 (Fig. 1) is known as the Amotape zone^{7,10,11}, and marks the northern limit of the central Andes⁸. The overall geology changes markedly at this point; to the north, a coastal belt of ophiolitic rocks appears^{8,12}, together with a line of recent stratovolcanoes associated with a longitudinal graben⁷. The boundary coincides with local transverse strikes⁸ and faults¹³, aligned with the Gulf of Guayaquil and the Carnegie Ridge to the west, and the Amazon depression, perhaps an old shield lineament¹¹, to the east.

Boundary 2 approximately coincides with the Huancabamba deflection¹⁴ at a point where the Andes change their strike from north-west to north-north-east.

Transverse strikes have been noted on boundary 3 (ref. 10), which marks the southern limit of the Coastal Cordillera.

Boundary 4 coincides with the Pisco¹⁴ or Abancay^{15,16} deflection and has been proposed as a line of division in the central Andes⁸. It is marked by a major step in the coastline, which indicates the abrupt northward disappearance of the Precambrian formations of the Coastal Cordillera^{17,18}, and by the offshore Nazca Ridge, and a shallowing of the Peru–Chile trench¹⁹. On the boundary there is a marked northward narrowing and change in direction of the Eastern Cordillera, and north-east–south-west striking formations^{16,20}. Important changes in the Mesozoic palaeogeography have also been observed¹⁶.

Boundary 5 is marked by the northern limits of the belt of recent volcanoes and the Altiplano–Puna block.

Boundary 6 is characterised by a change in Andean strike, from north to north-west, and by a northward narrowing of the Eastern Cordillera, where it has been termed the Ichilo fault zone²¹ or line²², or the Arica Elbow line²³. East of the Andean orogen the Precambrian basement is veneered by Cainozoic sediments to the north of the boundary, whereas to the south, Palaeozoic and Cretaceous formations are also present²⁴. The

westward extension of the Arica Elbow line is less apparent but has been proposed^{22,23,25}.

Boundary 8 is so positioned because of the coincidence of a sinuous portion of the Peru–Chile trench¹⁹ with the increase in width of the longitudinal valley (Fig. 1), the northern limit of the north-north-east trending Cordillera Domeyko²⁶, a westward step in the longitudinal belt of recent volcanoes, and an extension of Quaternary volcanoes in an irregular transverse belt extending as far as the Eastern Cordillera, especially in Sud Lipez in southernmost Bolivia.

Boundary 10, between the Norte Grande and the Norte Chico, coincides with the approximate southern limits of the belt of recent volcanoes, the longitudinal valley and the elevated

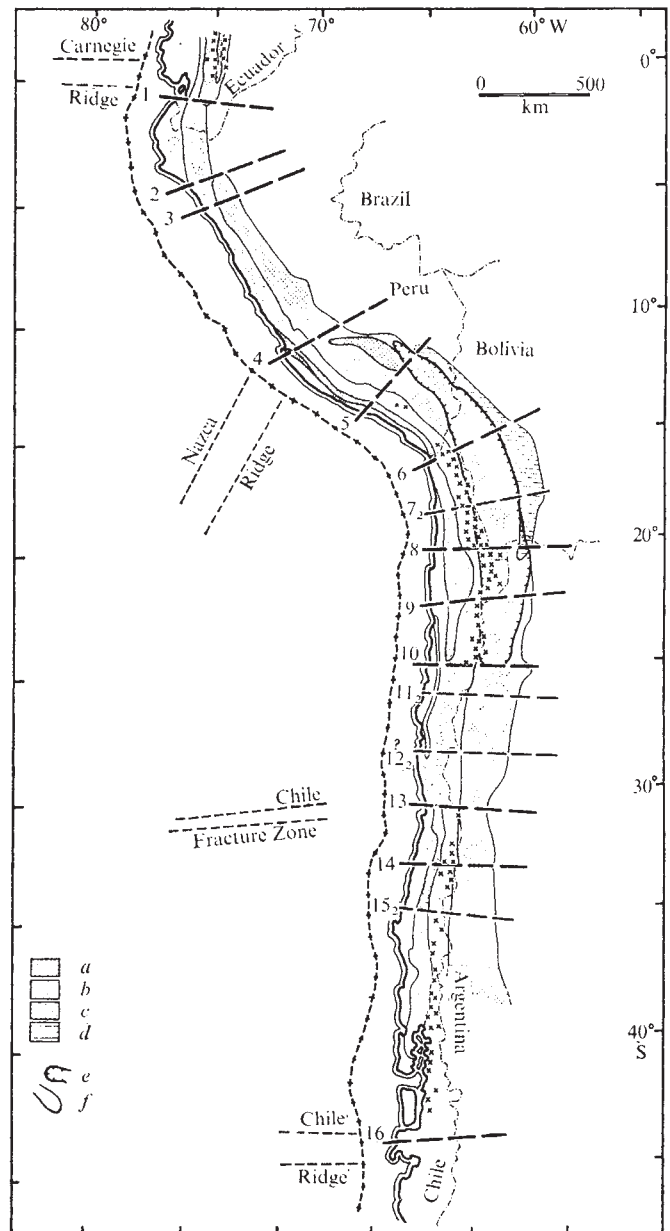


Fig. 1 The tectonic boundaries proposed for the central Andean volcano-plutonic orogen, in relation to certain physiographic features. *a–d*, metallogenic belts: *a*, iron; *b*, copper–(gold–molybdenum); *c*, copper–lead–zinc–silver; *d*, tin–(tungsten–silver); *e*, Altiplano–Puna; *f*, Longitudinal and Central Valleys; *x*, Location of recent volcanoes; +–+–+–, axis of Peru–Chile trench. Heavy dashed lines are the boundaries between the tectonic segments; numbered as referred to in the text; a subscript 2 denotes a boundary of only secondary importance.

Altiplano-Puna block²⁷ (Fig. 1). To the south there is a distinctive Cainozoic geomorphological development^{28,29}. The boundary also seems to have controlled the southern limit of widespread volcanism in the Jurassic, and the northern limit of widespread submarine volcanism in the Lower Cretaceous³⁰.

Boundary 13 marks the junction between Central Chile and the Norte Chico. It limits the northernmost extent of the belt of recent volcanoes, of the Central Valley—a Palaeogene feature^{31,32}, of the belt of Upper Tertiary andesitic-basaltic volcanics, and of widespread Precambrian rocks and Palaeozoic intrusives in the Coastal Cordillera²⁶. In Argentina, the boundary controls the southernmost extent of the Precordillera³³ and the Sierra de Córdoba. The boundary also lies at approximately the same latitude as the Chile fracture zone in the east-central Pacific (Fig. 1).

Boundary 16 is the southern limit of the central Andes⁸ and of the Central Valley of Chile (Fig. 1), and lies at the same latitude as the spreading Chile Ridge. It also corresponds to the southern limit of volcanism in Upper Cretaceous, Eocene, late Tertiary and recent time³⁰.

Correlations with seismicity

In order to confirm whether or not the present seismic boundaries in the Andes coincide with the geologically defined boundaries suggested here, and in an attempt to recognise additional boundaries, a study of the latitudinal distribution of intermediate and deep focus earthquakes is necessary. Some information on seismic provinces is, however, available and correlates reasonably well with the tectonic segmentation proposed here. Gajardo and Lomnitz⁵ defined provinces in Chile according to the levels of seismicity measured during a 16 yr period. Some of their boundaries correspond to boundaries 6, 8, 10, 14 and 15 (Fig. 1). Stauder³⁴ defined a similar series of boundaries between distinct seismic provinces, which correspond well to boundaries 6, 9, 10, 14 and 16 (Fig. 1). He also postulated that "the lithospheric slab" is apparently "segmented into a series of tongues that are absorbed independently". South of boundary 15 there is a shift of major seismicity to an offshore position^{31,35}. Kelleher³⁶ studied the rupture pattern of the shallow part of the seismic zone beneath the Andes. He determined that the zone between boundaries 1 and 3 (Fig. 1) has been aseismic this century, whereas that between boundaries 3 and 5 has experienced large earthquakes, and that between boundaries 13 and 16 has ruptured about once each century along its entire length. He also recognised the intervals between boundaries 5 and 9 and 9 and 13 as distinct seismic provinces.

Nature of tectonic boundaries

As favoured by Carr *et al.*⁹ for the Japanese arcs, I believe that the surface geological discontinuities in the central Andes probably correlate with subjacent seismic discontinuities, and reflect transverse boundary zones between separate segments of oceanic lithosphere which are subducted as individual units, perhaps even at different rates. The segmentation of the subduction zone is presumed to result from stresses created by the underthrusting of a cap-like slab of lithosphere, and the pattern produced may well be self-perpetuating. Transform faults, which divide up the oceanic lithosphere into a series of strips, may also play a part in the production of the segments. Shield lineaments could also be involved at the boundaries at which changes in Andean strike are apparent⁸, but they probably exert only a subsidiary influence.

The surface manifestations of the tectonic boundaries suggest that the magnitudes, and perhaps the histories, of their activity

are different. Furthermore, some boundaries (such as No. 13) seem to be fairly abrupt, and others (such as No. 10) more diffuse. It is not clear whether tectonic boundaries are relatively permanent features or whether they tend to migrate slowly or jump around with time. The fact that some of the present boundaries apparently occupied the same positions in early Cainozoic and even Mesozoic times, however, implies some degree of stability, or at least of repeated reactivation.

Although the discontinuities at the surface and on the subduction zone probably coincide, it does not necessarily mean that they are connected by major fault zones. Rather, I believe that the crustal segments overlying the various segments of the sinking slab have been subjected to similar, but clearly distinct, tectonic, geomorphological, magmatic and metallogenic regimes, with zones of transition between them. Consequently, the wholesale extrapolation of geological histories along the lengths of volcano-plutonic orogens should be avoided.

Implications for magmatism

Across the central Andes, post-Triassic calc-alkaline magmas, of probable subduction zone origin^{1,2,37,38}, have been emplaced episodically upon and within the continental crust as a series of discrete pulses^{30,39,40}. Each magmatic pulse has given rise to long, narrow belts of intrusive and extrusive rocks roughly parallel to the continental margin. Successive belts young eastwards in the western and central parts of the orogen^{30,39,40}, as is common in volcano-plutonic orogens⁴¹, whereas little lateral migration of magmatism took place in a second locus of activity along the eastern margin of the Andes at latitudes 14°–24°S.

Radiometric age data^{30,40,42}, together with the mapped distribution of the magmatic belts both in the west and centre of the orogen and further east, support the concept that the belts are not longitudinally continuous. Therefore, magmatism in any particular tectonic segment can be expected to possess its own unique episodicity and spatial distribution, replaced by similar but clearly distinguishable regimes to the north and south. Clear discontinuities in belts of intrusive rocks have been recognised at boundaries 2, 3, 4, 5, 6, 7, 8, 10, 13 and 14, and volcanism, particularly recent activity, only occurs in certain segments at any one time.

Although magmatism in the North American Cordillera is locally episodic, it is essentially continuous if viewed over the full extent of the orogen⁴³. Mesozoic-Cainozoic intrusive epochs cannot be correlated throughout the orogen⁴⁴. This may be simply explained if it is accepted that age patterns are only valid within individual tectonic segments.

Implications for metallogeny

The majority of the principal ore deposits in the central Andes have an inherent spatial and temporal relationship with the magmatic activity, and they are believed to derive their metal contents from the underlying subduction zone^{45–47}. Thus, it might be expected that the regional metallogeny varies between segments. An examination of the distribution of post-Palaeozoic metallogenic belts in the central Andes (Fig. 1, and ref. 46) reveals a coastal belt rich in iron, bordered to the east by a copper-(gold-molybdenum) belt, a copper-lead-zinc-silver belt, and, in the central part of the region, a tin-(tungsten-silver) belt. When tectonic boundaries are crossed these metallogenic belts tend either to end or to undergo changes in their characteristics, including: second-order variations in their metal contents; changes in their widths; and changes in the ages, types and sizes of deposits. The tin belt is restricted to three segments enclosed by boundaries 5 and 8 (Fig. 1), and is subdivided into a narrow, northern segment dominated by vein-type tin-tungsten deposits related to Mesozoic and

early Miocene batholiths, and a broader, southern part in which tin and tin-silver deposits of vein and porphyry¹⁹ types are largely related to late Tertiary subvolcanic stocks⁵⁰⁻⁵². Contact-metasedimentary and vein type iron deposits are located in the coastal zone between boundaries 1 and 2, 4 and 6, and 9 and 12 (refs 30, 53, 54). The copper belt extends northwards from boundary 15 (ref. 53), but loses a good deal of its importance north of boundary 4. The polymetallic province is particularly enriched in silver north of boundary 1 (ref. 13), but is most important, despite its narrowness, from boundary 2 to boundary 4. From boundary 4 to boundary 5 the metal content is anomalous and lead, zinc and silver are overshadowed by copper and iron (Fig. 1, and ref. 54), and south of boundary 8 mineralisation is rather sparse and dominated by copper⁵⁵. Within the copper belt the termination of long lines of distinctive ore types is also controlled by tectonic boundaries. Examples are the chalcopyrite-magnetite-actinolite veins that are known between boundaries 8 and 12 (ref. 53) and the disseminated, stratiform (manto-type) copper deposits in Jurassic volcanics from boundary 8 to boundary 10 (ref. 56). The porphyry copper deposits in the central Andes decrease in age eastwards³⁸ but, in addition, the major deposits change in age along the strike, so that Palaeocene deposits are dominant between boundaries 5 and 6 (refs 40 and 57), late Eocene-Oligocene deposits are dominant between boundaries 7 and 10, and late Miocene-Pliocene deposits are dominant between boundaries 12 and 14 (ref. 58). It is concluded that the definition of metallogenic segments is an important facet of the metallogenic characterisation of volcano-plutonic orogens and effectively explains the longitudinal variability of their metallogeny.

Mineral exploration

The mapping of metallogenic segmentation in both young and ancient volcano-plutonic orogens is clearly of paramount importance in the planning of mineral exploration programmes since metal contents, ore types and even economic importance change at tectonic boundaries.

Although a conclusive statement cannot be made at present, there is evidence from the central Andes that some major ore deposits tend to be located on or close to tectonic boundaries; the Chuquicamata and Río Blanco-Disputada porphyry copper deposits lie on boundaries 8 and 13, and the Oruro and Potosí porphyry tin deposits on boundaries 6 and 7. If magmas and their related metals are derived from the vicinity of the subduction zone, then this possibility seems to be more reasonable than the proposal that the locations of major ore deposits are controlled by megalineaments and their intersections (ref. 59 and 60), especially as lineaments in the central Andes are commonly at high angles to the tectonic boundaries⁶¹⁻⁶³ and are relatively superficial flaws in the upper continental crust.

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¹ James, D. E., *Bull. geol. Soc. Am.*, **82**, 3325-3346 (1971).

² Dickinson, W. R., *Rev. Geophys. Space Phys.*, **8**, 813-860 (1970).

³ Brüggen, J., *Fundamentos de la Geología de Chile* (Inst. Geogr. Milit., Santiago, 1950).

⁴ Muñoz Cristi, J., in *Handbook of South American Geology* (edit. by Jenks, W. F.), 187-214 (*Mem. geol. Soc. Am.*, **65**; 1956).

⁵ Gajardo, E., and Lomnitz, C., *Proc. 2nd World Conf. Earthquake Engng*, **3**, 1529-1540 (1960).

⁶ Lomnitz, C., *J. geophys. Res.*, **67**, 351-363 (1962).

⁷ Gerth, H., *Der Geologische Bau der südamerikanischen Kordillere* (Borntraeger, Berlin, 1955).

⁸ Gansser, A., *J. geol. Soc. Lond.*, **129**, 93-131 (1973).

⁹ Carr, M. J., Stoiber, R. E., and Drake, C. L., *Bull. geol. Soc. Am.*, **84**, 2917-2930 (1973).

¹⁰ Steinmann, G., *Geología de Perú* (Carl Winters Universität, Heidelberg, 1930).

¹¹ de Loczy, L., *An. Acad. brasil. Cienc.*, **42**, 185-205 (1970).

¹² Goossens, P. J., and Rose, W. I. jun., *Bull. geol. Soc. Am.*, **84**, 1043-1052 (1973).

¹³ Goossens, P. J., *Econ. Geol.*, **67**, 458-468 (1972).

¹⁴ Ham, C. K., and Herrera, L. J. jun., in *Backbone of the Americas* (edit. by Childs, O. E., and Beebe, B. W.), 47-61 (*Mem. Am. Ass. Petrol. Geol.*, **2**; 1963).

¹⁵ Jenks, W. F., in *Handbook of South American Geology* (edit. by Jenks, W. F.), 215-247 (*Mem. geol. Soc. Am.*, **65**; 1956).

¹⁶ Marocco, R., *Cah. ORSTOM, sér. Géol.*, **3**, 1, 45-58 (1971).

¹⁷ Bellido, E., *Bol. Serv. Geol. Min., Lima*, **22** (1969).

¹⁸ Cobbing, E. J., and Pitcher, W. S., *Nature phys. Sci.*, **240**, 51-53 (1972).

¹⁹ Fisher, R. L., and Raitt, R. W., *Deep-Sea Res.*, **9**, 423-443 (1962).

²⁰ Ruegg, W., *Bol. Soc. Geol. Perú*, **38**, 97-142 (1962).

²¹ Rod, E., *Bull. Am. Ass. Petrol. Geol.*, **44**, 107-108 (1960).

²² Radelli, L., *Trav. Lab. Geol. Grenoble*, **42**, 237-261 (1966).

²³ Schlatter, L. E., and Nederlof, M. H., *Bol. Serv. geol. Bolivia*, **8** (1966).

²⁴ Ahlfeld, F., *Geol. Rdsch.*, **59**, 1124-1140 (1970).

²⁵ Sonnenberg, F. P., in *Backbone of the Americas* (edit. by Childs, O. E., and Beebe, B. W.), 36-46 (*Mem. Am. Ass. Petrol. Geol.*, **2**; 1963).

²⁶ *Mapa Geológico de Chile*, 1:1,000,000 (Inst. Invest. Geol., Santiago, 1968).

²⁷ Turner, J. C. M., *Geol. Rdsch.*, **59**, 1028-1063 (1970).

²⁸ Sillitoe, R. H., Mortimer, C., and Clark, A. H., *Trans. Inst. Min. Metall.*, **B77**, 166-169 (1968).

²⁹ Mortimer, C., *J. geol. Soc. Lond.*, **129**, 505-526 (1973).

³⁰ Ruiz, F. C., Aguirre, L., Corvalán, J., Kohn, C., Kohn, E., and Levi, B., *Geología y Yacimientos Metalíferos de Chile* (Inst. Invest. Geol., Santiago, 1965).

³¹ Levi, B. D., Aguilar, A. M., and Fuenzalida, R. P., *Bol. Inst. Invest. Geol., Santiago*, **19** (1966).

³² Carter, W. D., and Aguirre, L., *Bull. geol. Soc. Am.*, **76**, 651-664 (1965).

³³ Herrero-Ducloix, A., in *Backbone of the Americas* (edit. by Childs, O. E., and Beebe, B. W.), 16-28 (*Mem. Am. Ass. Petrol. Geol.*, **2**, 1963).

³⁴ Stauder, W., *J. geophys. Res.*, **78**, 5033-5061 (1973).

³⁵ Lomnitz, C., *Geol. Rdsch.*, **59**, 938-960 (1970).

³⁶ Kelleher, J., *J. geophys. Res.*, **77**, 2087-2103 (1972); Kelleher, J., et al., *J. geophys. Res.*, **78**, 2547-2585 (1973).

³⁷ Dickinson, W. R., *Am. J. Sci.*, **272**, 551-576 (1972).

³⁸ Sillitoe, R. H., *Econ. Geol.*, **67**, 184-197 (1972).

³⁹ Farrar, E., Clark, A. H., Haynes, S. J., Quirt, G. S., Conn, H., and Zentilli, M., *Earth planet. Sci. Lett.*, **10**, 60-66 (1970).

⁴⁰ Stewart, J. W., Evernden, J. F., and Snelling, N. J., *Bull. geol. Soc. Am.* (in the press).

⁴¹ Dickinson, W. R., *J. geophys. Res.*, **78**, 3376-3389 (1973).

⁴² Clark, A. H., and Farrar, E., *Econ. Geol.*, **68**, 102-106 (1973).

⁴³ Gilluly, J., *Bull. geol. Soc. Am.*, **84**, 499-514 (1973).

⁴⁴ Lanphere, M. A., and Reed, B. L., *Bull. geol. Soc. Am.*, **84**, 3773-3782 (1973).

⁴⁵ Sillitoe, R. H., *Bull. geol. Soc. Am.*, **83**, 813-818 (1972).

⁴⁶ Sillitoe, R. H., *Geol. Assoc. Canada Spec. Pap.* (in the press).

⁴⁷ Sawkins, F. J., *J. geophys. Res.*, **80**, 377-397 (1972).

⁴⁸ Petersen, U., *Geol. Rdsch.*, **59**, 834-897 (1970).

⁴⁹ Sillitoe, R. H., and Halls, C., submitted for publication.

⁵⁰ Ahlfeld, F., and Schneider-Scherbina, A., *Los Yacimientos Minerales y de Hidrocarburos de Bolivia* (Depto. Nac. Geol. Bol. 5, La Paz, 1964).

⁵¹ Ahlfeld, F., *Miner. Deposita*, **2**, 291-311 (1967).

⁵² Turneure, F. S., *Econ. Geol.*, **66**, 215-225 (1971).

⁵³ Ruiz, F. C., and Erickson, G. E., *Econ. Geol.*, **57**, 91-106 (1962).

⁵⁴ Bellido, E., and de Montreuil, L., *Aspectos Generales de la Metalogenia del Perú* (Serv. Geol. Min., Geol. Econ. 1, Lima, 1972).

⁵⁵ Angelelli, V., Fernandez Lima, J. C., Herrera, A., and Aristarain, L., *Descripción del mapa Metalogenético de la República Argentina. Minerales Metalíferos* (Direc. Nac. Geol. Min., Buenos Aires, 1970).

⁵⁶ Ruiz, C., Aguilar, A., Egert, E., Espinosa, W., Peebles, F., Quezada, R., and Serrano, M., in *Proc. IMA-IGOD Meetings '70, IAGOD Vol.* (edit. by Takeuchi, T.), 252-260 (Soc. Mining Geol. Japan Spec. Issue 3, Tokyo, 1971).

⁵⁷ Laughlin, A. W., Damon, P. E., and Watson, B. N., *Econ. Geol.*, **63**, 166-168 (1968).

⁵⁸ Quirt, S., Clark, A. H., Farrar, E., and Sillitoe, R. H., *Geol. Soc. Am. Abstr. with Prog.*, **3**, 7, 676-677 (1971).

⁵⁹ Mayo, E. B., *Min. Engng.*, **10**, 1169-1175 (1958).

⁶⁰ Thomas, N. A., *Geol. Rdsch.*, **59**, 1013-1027 (1970).

⁶¹ Segerstrom, K., *Prof. Pap. US geol. Surv.*, **700-D**, 10-17 (1971).

⁶² United Nations, *Fotolineamientos y Mineralización en el Noroeste Argentina* (Exploración Minera de la Region Noroeste, Argentina, inf. tec., New York, 1973).

Fig. 1 The sequence of yeast tRNA^{Phe} arranged in the clover leaf formula²¹. An arm of the clover leaf is made up of a double helical stem and a single stranded loop. Bases which are invariant in all tRNA sequences²² are circled; semi-invariants, that is, purines or pyrimidines exclusively, are bracketed. Nucleotides which are base paired in the tertiary structure are joined by solid lines, and those which stack on each other by dashed lines.

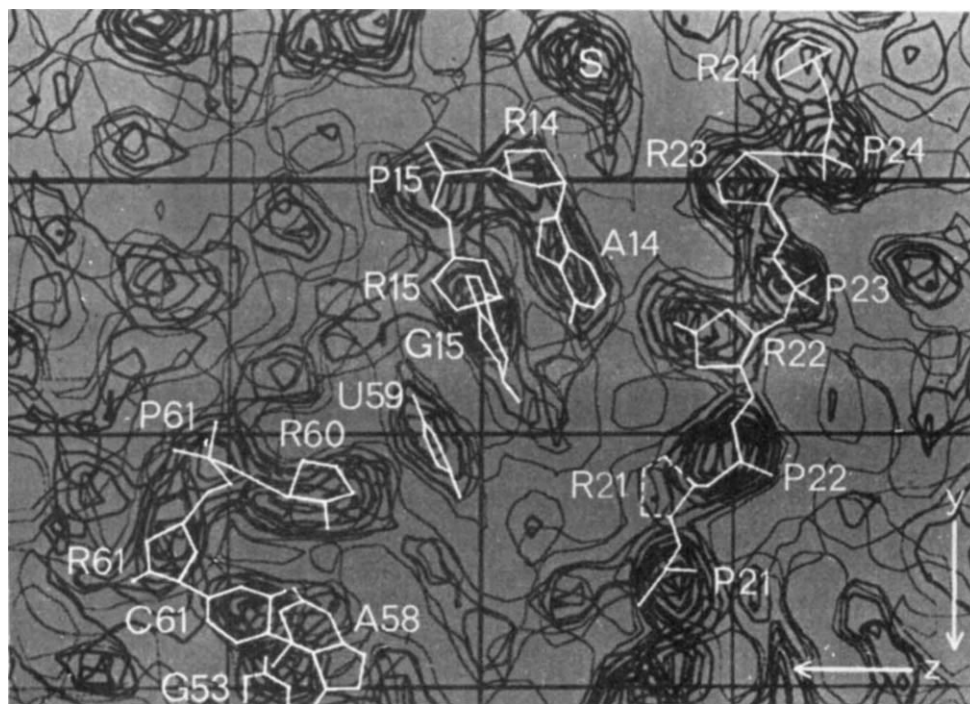


Fig. 2 A composite of four electron density map sections at $x=48-51$ Å. This includes a strand of the D stem. Most of the electron density not identified belongs to adjacent molecules or to cations. The heavy white lines represent the skeleton of the molecule. R21 lies just outside the sections shown. P: phosphate group; R: ribose; S: probably spermine. The grid squares are of side 10 Å. This region corresponds to part of Fig. 4 of the MIT paper¹.

size and shape to be identified and hence related to the amino acid sequence. In contrast, in RNA there are essentially only two types of residue, purine and pyrimidine, not easily distinguishable from each other. Moreover the bases have a total mass similar to the phosphates and riboses, and, when base-paired to bridge two pieces of backbone not in a double helical region, they give that part of the map more the character of a network than of a dominant single chain standing out from a lower landscape. The situation is further complicated by the presence of numerous cations, Mg^{2+} , Na^+ or spermine, many hydrated and held in fixed positions. In tracing the ribose phosphate chain from the electron density map, the most important constraint comes from the expectation of finding the four double helical stems of the clover leaf formula and the necessity of joining them correctly. The electron density corresponding to the chain was generally clear in the double helical regions. In most areas clearly resolved peaks could be seen for each ribose and for each phosphate, which could usually be distinguished by their shape and by the connectivity of the density: the phosphate groups tend to be spherical while ribose density is more disk-like. The angles made between the groups are also very characteristic, giving a distinct sugar-phosphate stagger which is difficult to misinterpret (Fig. 2). Fitting the model in such regions fixed the polarity of the chain unambiguously.

The assignment of the helical stems could be checked because in some base pairs the purine can be distinguished from the pyrimidine, and wherever a decision can be made, the result agrees with the sequence (Fig. 3a). Confirmation of the helix assignment comes from chemical work on the platinum derivative. Fingerprints of various enzyme digests of the tRNA reacted with the platinum compound showed that the metal was bound in the fragment 34 to 39, agreeing with the position found for it in the model.

In regions of the map which do not contain double helical segments, the connectivity and polarity of the chain is more difficult to determine from electron density alone. In some places the thermal motion or disorder smears out the phosphate or ribose peaks. Here it is important to use conformational information^{7,8} obtained from single crystal structure analysis of oligonucleotides. In the end we were able to trace the whole chain except for several nucleotides

in the D and T ψ C loops. These lie close together in one corner of the molecule and are also in tight contact with the same loops of two other symmetry-related molecules. Here the density is strong but broken up and we have not been able to arrive at an unambiguous chain tracing. The stretches in question are however confined to a small region of the map so that, whatever the final details, their structure will be constrained within the bounds proposed in our model.

Table 1 Final heavy atom parameters and refinement statistics to 3 Å

Derivative	Site	Z	x	y	z	B	n	r.m.s. f_H	r.m.s. E	P
<i>trans</i> Cl ₂ (NH ₃) ₂ Pt	1	76.8	0.179	0.0	0.145	24.6	4,411	69.9	40.8	1.72
	1	58.7	0.629	0.147	0.453	17.4	2,090	86.9	59.5	1.46
	2	44.0	0.098	0.089	0.594	23.8				
	3	29.4	0.769	0.001	0.548	21.4				
Sm ³⁺	4	16.7	0.825	0.238	0.264	20.8				
	1	47.7	0.626	0.145	0.454	18.3	4,627	55.4	39.7	1.40
	2	31.7	0.098	0.091	0.594	26.9				
	3	9.7	0.768	-0.008	0.554	19.3				
Os-ATP complex ^{23,24}	4	9.1	0.828	0.243	0.264	25.5				
	32.7	0.236	0.446	0.974	36.2	4,338	42.9	37.9	1.14	
	2	29.1	0.177	-0.001	0.146	14.2				
	3	21.6	0.626	0.362	0.735	34.4				
Pt + Lu	Pt1	62.8	0.180	0.0	0.146	25.5	4,184	80.0	43.5	1.84
	Lu1	41.3	0.629	0.144	0.453	15.6				
	2	32.5	0.099	0.090	0.595	24.8				
	3	26.2	0.771	-0.005	0.548	35.9				

Z is the occupancy. B is the isotropic temperature factor. n is the number of reflections used in the refinement. f_H is the calculated heavy atom contribution to the structure factor. E is the closure error, $|F_H(\text{observed}) - F_H(\text{calculated})|$. P is the phasing power, the ratio of the two previous columns. The Os derivative was refined in three shells because of changing occupancy; the Z quoted is the mean. The mean figure of merit for 4,838 reflections = 0.67. The mean standard deviation in $|F|$ of the reflections measured more than once during data collection was 6% of the mean $|F|$ for the native crystal, and somewhat higher for the derivatives. Structure factors and intensities were calculated using hand-measured coordinates of residues 1-17, 21-54 and 58-76. The classical R-factor on $|F|$ s is 0.468 and the correlation coefficient between calculated and observed intensities is 0.567.

Since we were not always able to distinguish purines from pyrimidines even within helical regions, the sequence data were initially of little help in solving the atomic structure, but as the helical regions and sequence numbering became established, features of density corresponding to the bases became interpretable. For example, the electron density at base position 26 was of a shape consistent with a twice

methyated G, and so provided a valuable marker in the model building. Also, the shape of the electron density for the G15 base (Fig. 3b) gave us confidence that the base pair 15–48 was of the unorthodox type to be discussed below.

Some features of the cation distribution also began to emerge. An interesting example is an ion found chelated between the amino groups of G25 and G45 and the oxygens of phosphates 23 and 24. This may be one of the tightly bound magnesium ions which seem to be an integral part of the native structure⁹.

Molecular structure and tertiary interactions

Figures 4 and 5 are photographs of the molecular model. The anticodon is at the upper right, the amino acid stem at the upper left, with the T ψ C loop in the bottom left corner. The relative arrangement of the double helical segments is indicated schematically in Fig. 6 which also shows the topology of the connections made in the tertiary structure. (These tertiary interactions are also indicated in Fig. 1.) All but seven of the bases are represented in the diagram. Base pairs additional to those in the stems are shown as dotted lines. As might be expected¹⁰, they are protected from water by stacking or intercalation. The stacking and intercalation of unpaired bases is also indicated. The region shown dashed is the T ψ C loop–D loop corner where we have not been able to determine the structure unambiguously.

The amino acid stem is stacked on the T ψ C stem in such a way that a long double helix of 12 base pairs is formed in which one of the ribose phosphate chains (72–61) is not interrupted. In the companion strand there is a break between nucleotides 7 and 49, though this does not seem to disturb the overall perfection of the long double helix. But the sharp turn made at the 50–49–48 corner involves the close approach of phosphates (~ 5 Å), and the details are subject to revision at higher resolution. The G–U base pair 4–69 also seems to be made without any major disturbance to the helix, at least at this resolution. The long double helix is right handed, has its bases tilted to the axis, and the axial distance between residues 61 and 72 is 30 Å so that the repeat per residue along the helix axis is 2.8 Å. The helix is therefore of the A form. At one end of the helix are stacked the unpaired bases A73 and C74, and at the other end, T54 which is also paired to another base in the T ψ C loop.

In contrast to the amino acid and T ψ C stems, the D stem and the anticodon stems are only approximately collinear, lying 20° from each other. The two together, with nucleotide 26 in between, form a long imperfect double helix which lies roughly at right angles to the first long helix, and meets it at its middle, so that the two are arranged rather as the letter T.

The D stem is guyed at the T join to the long double helix by part of the variable loop (residues 44–48) and by the stretch 8–9 which connects one strand of the amino acid stem to one strand of the D stem. This stretch, which is two nucleotides long in all tRNAs, is fully extended in the model, allowing it to span the length of the D stem. A9 makes a base pair, of the type found in poly(A)¹², with A23, which is paired to U12 in the D stem, so that all three together form a base triple. A base pair of the reverse Hoogsteen type¹³ is formed between U8 and A14, both of which are invariant nucleotides. U8 is then stacked on C13 and in the right orientation to allow the photoreaction observed by Yaniv¹⁴. On the pair 8–14 is stacked an unusual base pair formed between G15 of the D loop and C48 of the extra loop (Fig. 3b). These two bases form the correlated pair noticed by Levitt¹⁵ and might have been expected to form a Watson–Crick pair. We discuss this question below.

Thus the D stem has been augmented along its length by the non-standard base pairs 8–14 and 15–48. On the end of

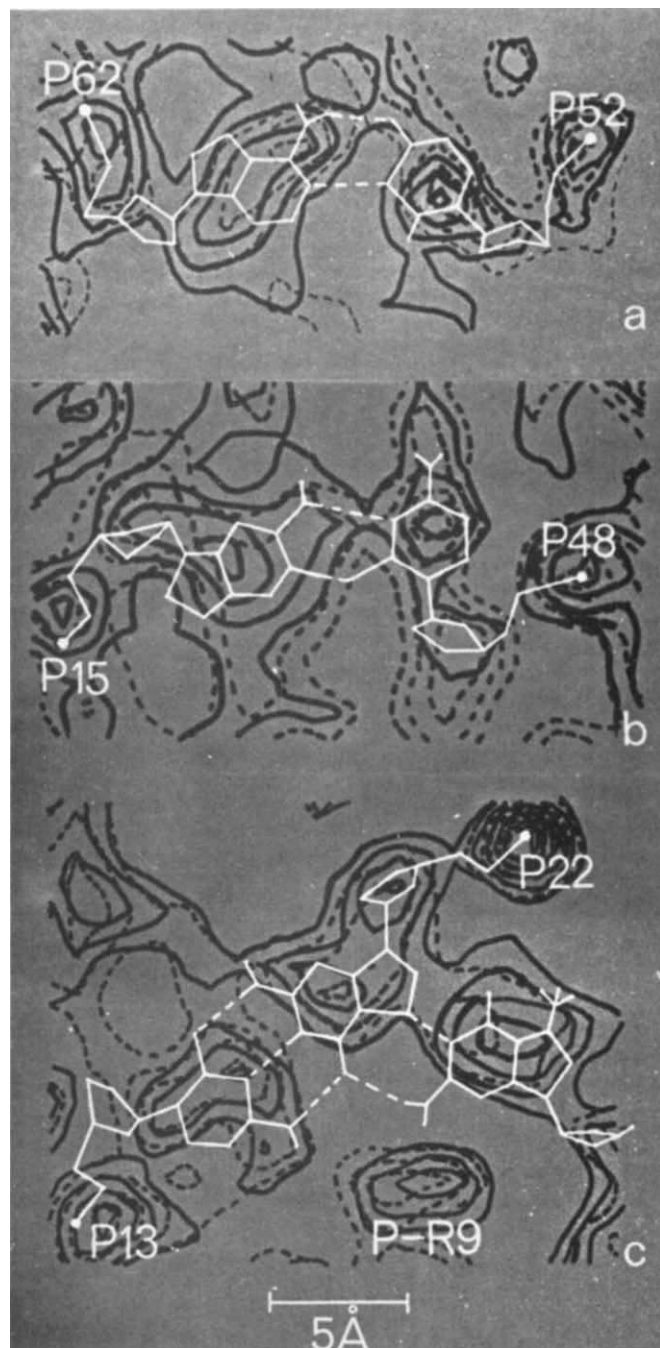


Fig. 3 Parts of skew slices of the electron density map showing the density distribution in the planes of certain base pairs or base triples. Each slice comprises two sections 1 Å apart (full and dotted contours). The superposed skeleton is taken from the coordinates of the unrefined model. The ribose groups generally lie out of the planes of the sections. *a*, A Watson–Crick base pair from the T ψ C stem. Additional peaks can be seen in the neighbourhood of non-bonded polar groups, probably water molecules or cations. *b*, The unusual base pair G15–C48 with the glycosyl bonds in the *trans* position. The peak above the amino group of C48 may be a cation or water molecule. *c*, A base triple from the augmented D helix, 13–22–46.

this again there is stacked a single base for which there is strong density coming up from the T ψ C loop. This is either U59 or A58 depending on the resolution of the ambiguity discussed below. This base seems to mediate the abutting of the augmented D stem on to the long double helix formed by the amino acid and T ψ C stems, by fitting into the large groove and acting as a sort of peg to help hold the two perpendicular helices together. Apart from the connection 15–48, the D arm is also connected to the extra arm at a further point: G46 is base paired to G22, so that there is a second base triple 46–22–13 (Fig. 3c) in the structure. At the other end of the D stem is stacked the unpaired base G26, so that finally the augmented D helix contains a stack eight base spacings long. The translation per residue is about 3 Å so that this helix is closer to the A' form of RNA¹¹.

Continuing around the D loop, nucleotides D16 and D17 arch outwards from the body of the molecule. The invariant pair of residues G18 and G19 come into close contact with the T ψ C loop in a manner to be described,

with the base of 26 lying in the bearing formed by 44 and 45. A result of the tilt is that the major groove of the long, imperfect double helical segment formed by the anticodon and D stems is now some 5 Å narrower than for the more perfect double helix formed by the amino acid and T ψ C stems.

The anticodon loop itself is stacked on the 3' side roughly in the manner suggested by Fuller and Hodgson¹⁷. The density is weak in this region so we cannot be confident of the position of the bases but it seems that base 38 points inwards, 37 more outwards and finally the three bases of the anticodon protrude outwards from a quasihelical backbone. Here O2' of ribose 35 comes close to O1' of ribose 36, suggesting a hydrogen bond, and there is a similar linkage between 36 and 37. We can see no special structural rôle for the Y base 37. On the 5' side of the loop the bases 32 and 33 are also stacked but seem to face inwards rather than outwards⁷. This is made possible by kinks in the backbone at the two residues 32 and 34 methylated in the O2' position.

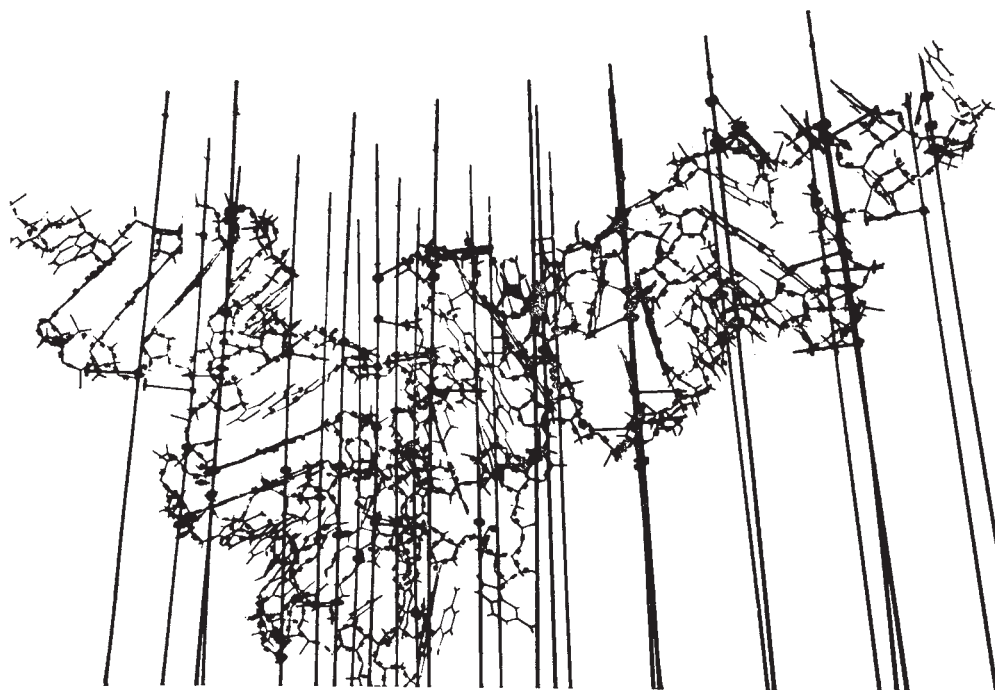


Fig. 4 A photograph of the model taken in a direction roughly perpendicular to the plane of the T made by the two long double helical segments (see Fig. 6). The geometrical shape of the T is somewhat obscured by the chains of the T ψ C and extra loops.

whereas G20, which is variable, comes up again close to the other variable nucleotides 16 and 17 and points outwards. The exposed position of these three residues is consistent with their chemical reactivity¹⁶. A21, which is invariant, stacks and intercalates between the base pairs 13–22 and 15–48. It lies in the plane of the base pair 8–14 but the density does not seem to permit another base triple here, tantalisingly close as it is. Possibly A21 forms a hydrogen bond with the ribose of 8. It may be that in other species of tRNA this would be a base triple formed by the three invariant bases, 8, 14 and 21.

The axis of the anticodon stem is not only tilted by about 20° to the D stem, but is also dislocated by about 5 Å from the line of the D stem. Thus the bases A44 and G45 are stacked on the last base pair 27–43 of the anticodon stem, but lie below the right hand end of the augmented D helix, with G26 partially intercalating between them. All tRNAs have a single unpaired base in position 26 and the structure suggests that this feature is associated with the tilting of the anticodon stem relative to the D helix. Indeed, the structure here presents the appearance of a hinge,

We have not been able to make an unambiguous tracing of the chain in the T ψ C loop region, but there are some features of which we are reasonably certain. T54 is paired to another base in the same loop, a base from this loop stacks on the end of the augmented double helix and G57 lies close to G18 and G19 of the D loop. We have found two alternative configurations, both of which are stereochemically acceptable. In the first version, T54 is Hoogsteen paired with A58, U59 stacks on G15, G57 intercalates between the bases of G18 and G19, and C56 is close enough to G18 or G19 to form a base pair. In the second version, the base which pairs with T54 is C60 and that which stacks on the augmented D helix is A58. The three bases G18, G19 and G57 still form a stack, but the order is now 18–19–57 rather than 18–57–19. This stacking proposed for the three invariant Gs is a novel and certainly unexpected tertiary interaction, but it remains to be proved correct. In both versions G20 is in the accessible part of the D loop, in the neighbourhood of D16 and D17.

At the 3' end, A73 and C74 are stacked on the end of the amino acid stem but the final ACCA stretch swings

across the unit cell and packs between the anticodon stems of two other symmetry related molecules. These latter stems are 33 Å apart in the *y* direction and the gap is nicely filled by this ACCA chain from the first molecule.

Relation to the MIT model

The relationship between the monoclinic form and the orthorhombic form studied⁴ at MIT is *a, b, c* (MRC) → *b, -a, c* (MIT). In view of the similarity of X-ray intensities⁷ the contents of half the orthorhombic unit cell should superpose on the whole monoclinic cell when aligned on the common 2₁ axes. We have found the relative translation from the lanthanide positions common to the two crystals, and the map sections published by the MIT group⁴ are generally similar to the corresponding sections in our map, but there are everywhere significant differences in detail and major differences in interpretation.

The overall shape of our model is similar to that proposed by the MIT group and we seem to agree about the relative positions of the anticodon, amino acid and TψC stems, but there is complete disagreement about the orientation of the central D stem. This difference means that almost all the connections to the remainder of the molecule must be radically different in the two models. We have been able to define a number of tertiary interactions in the molecular centre holding it rigid, whereas the only tertiary interaction proposed in the MIT model is that between the correlated base pair 15-48 which is, by implication, of the Watson-Crick type, contrary to our findings. In our model the D helix lies approximately normal to the TψC stem, that is, parallel to the long *c* axis, and the base pairs lie in planes roughly perpendicular to *c* (Fig. 4). The density corresponding to the bases is quite unequivocal in this respect. But in

(MIT Fig. 5) the sugar-phosphate stagger and the bases have not been fitted correctly to the density. It is presumably these misfittings which have led the MIT workers to state⁴ that the double helices in the stems are not regular and cannot be assigned to one of the standard classes. We find helices which are regular at the resolution of the map.

There seem to be many other differences between the two structures, though they mostly involve the loop regions, where the MIT chain tracing is presented as tentative. Thus, in the MIT model the large Y base 37 extends across the major groove between the anticodon and D double helices to touch a phosphate on the D stem, whereas we have found the Y base positioned inside the anticodon loop. The structure of the anticodon loop is not described in the MIT paper, but from the picture of their model it is clearly not the ordered structure which we have built. Likewise, the interactions formed by the extra loop in our model do not seem to be made in the MIT model, and no others are described. In the MIT model, many of the bases in the TψC loop and the distal part of the D loop seem to be exposed, whereas in our model, despite the ambiguity mentioned earlier, they are stacked or otherwise not accessible to chemical attack, as is observed¹⁶.

Conclusions

The molecule can be described as consisting of three major substructures (Fig. 6). These are: (1) the long double helix formed by the amino acid and TψC stems stacking on top of each other; (2) the augmented D helix, forming the "thorax" of the molecule: this consists of the D stem augmented by part of its own loop and interlocked with both the stretch 8-9 and with part of the extra loop; (3) the anticodon stem, tilted off by about 20° at the other end

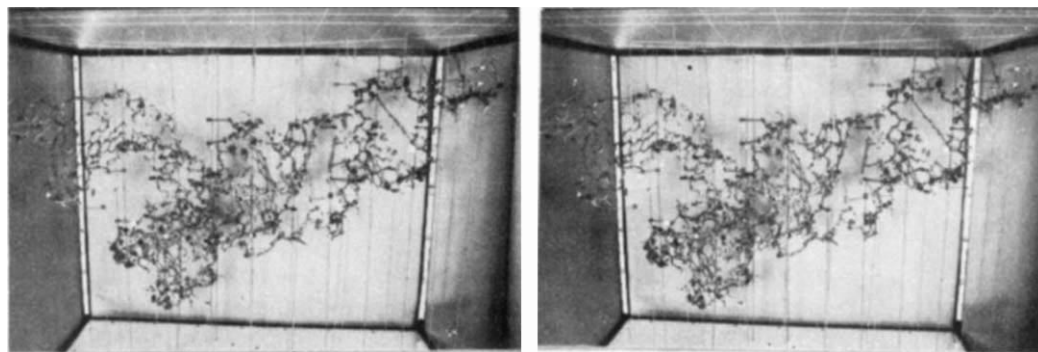


Fig. 5 A stereophotograph of a Kendrew skeletal model (2 cm per Å) of yeast tRNA^{Phe} built to fit the 3 Å map. The amino acid stem is at the upper left, and the anticodon at upper right. The TψC loop and distal part of the D loop are at the bottom left. The model is viewed approximately in the +*x* direction with +*z* running approximately from right to left. The whole CCA end at upper left is not in the frame of the photograph.

the MIT model the direction of the D stem is roughly perpendicular to *c*, as can be seen in the MIT Fig. 2 and from the disposition of the base pair 13-22 in their Fig. 4. Likewise the connecting sequence 8 and 9 runs horizontally in our model and more or less vertically in the MIT model.

Comparing our Fig. 4 with the corresponding area in Fig. 4 of the MIT paper, one sees that the only assignment here common to the two models is ribose 61. The MIT model has the base pair 13-22 placed in a way quite inconsistent with our density map and in effect forces a connection between residues 15 and 23. The assignment of the nucleotide sequence on the D stem is thus incorrect by two residues on one strand and by one on the other. We note that the other double helical stems have also not been fitted correctly in the MIT model²³. Thus, the MIT workers have placed R26 (their Fig. 3) where we have R27, so that the phase of the nucleotide sequence is incorrect and the base pair 42-28 is wrongly fitted to the density. This means that the tilt of all the bases in the anticodon helix must be wrong. Likewise we find that in the TψC helical stem

of the D stem and possibly hinged to it. These three main helical directions correspond well to the three largest peaks in Levitt's search of the X-ray intensities of the native crystal¹⁸: (1) corresponds to the peak at (−60°, −35°), (2) to (−15°, 0°), and (3) to (−35°, 0°), where the first value gives the rotation angle about *a*, and the second about *b*. The angle between (1) and (2) is then 103°.

To these substructures are adjoined other parts with clear functional rôles: (4) the ACCA sequence, at one end of (1), to carry the amino acid; (5) at the other end, the almost invariant TψC loop tightly folded and connected to the two invariant Gs 18 and 19 of the D loop, probably forming a ribosomal recognition site common to all tRNAs. The most variable nucleotides in the D loop, 16, 17 and 20, form (6) a patch flanking the augmented D helix (on the far side in Fig. 5); this may be an enzyme discrimination site, different for different tRNAs. There is also the region (7), on the other side of the D helix here consisting of a few variable bases; this can be assigned to the large extra arm which is present in other classes of tRNA, presumably for discrimi-

nation. Finally there is (8), the anticodon loop carrying the anticodon itself. This loop is stacked on the 3' side of the anticodon stem, but it has been suggested to us by Dr F. H. C. Crick that, if indeed the join between the anticodon stem and the augmented D helix functions as a hinge, then the conformation of the loop might possibly alter during protein synthesis, in the manner proposed by Woese¹⁹.

The unusual base pair 15-48 requires some discussion. Levitt¹⁵ noticed that 15 is always either G or A with 48 respectively C or U. There is only one exception²⁰ to this rule so far observed, in glycine tRNA where the bases are A and C. It is at first sight rather surprising that the nucleotides in these positions do not form a standard base pair. But if they did, one might expect the purine and pyrimidine to be exchanged between the two positions in some tRNAs. The fact is that the purine is always in position 15 and the pyrimidine always in 48. This speaks

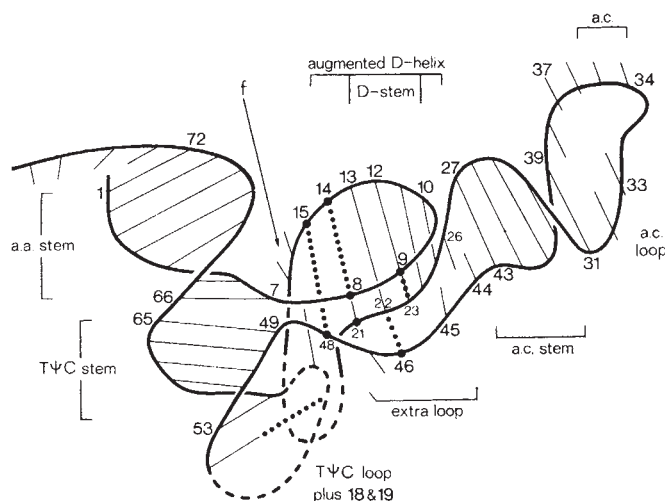


Fig. 6 A schematic diagram of the tertiary structure of yeast tRNA^{Phe}. The ribose-phosphate backbone is represented by a continuous line, except where there is ambiguity when it is shown dashed. Base pairs in the double helical stems are represented by long light lines, and non-paired bases by shorter lines. Many of the latter stack as indicated; those which do not are drawn at an angle, for example 16, 17 and 47. Base pairs additional to those in the clover leaf formula are indicated by dotted lines. The region marked f is explained in the text.

for an asymmetry in the bonding between the two chains, such as we have described. It is not possible to interchange the purine and pyrimidine and maintain the same disposition of the glycosyl bonds. Moreover, a 'reverse Watson-Crick' A-U pair can be accommodated here with almost the same *trans* arrangement of glycosyl bonds. It would seem that this nonsymmetrical pair of glycosyl bonds is required by the particular demand of connecting two locally parallel chains not related by any kind of dyad. Models of all ten combinations of base pairs in which the glycosyl bonds are *trans* to each other show that the two pairs G-C and A-U are the most similar, distinct from all the other possibilities.

We believe that our model can accommodate all tRNA sequences of the class I type¹⁵, and that many of its features will be retained in the other two classes. Class I has four bases in the D stem and five nucleotides in the extra loop, and all sequences can be fitted into the model without rearrangement. Most sequences have C13, G22 and G46 and hence can make the base triple 13-22-46. One of the remaining species has U13-A22, but 46 is now an A, and this combination can form a base triple with the three glycosyl bonds in the same positions. We thus have another

example of a set of correlated base changes. Most class I sequences also have U12, A23 and A9 and can make the second triple base described above. There is a subclass which has G12, C23 and G9, but G9 can make only one hydrogen bond to C23, unless there is a different tautomeric form or adopts a syn conformation. There is always the possibility of a third triple interaction using the invariants U8-A14-A21 as mentioned above.

We should like to emphasise that the model is in good accord with the results of a companion study of the chemical reactivity of yeast tRNA^{Phe} as already reported by us²⁶. The model is stereochemically sound within the physical limits of building with skeletal wire components, and is now being refined. Data to higher resolution are also being collected.

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Note added in proof: The MIT workers have now revised their model (Rich, personal communication).

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- Clark, B. F. C., Doctor, B. P., Holmes, K. C., Klug, A., Marcker, K. A., Morris, S. J., and Paradies, H. H., *Nature*, **219**, 1222-1224 (1968).
- Brown, R. S., Clark, B. F. C., Coulson, R. R., Finch, J. T., Klug, A., and Rhodes, D., *Eur. J. Biochem.*, **31**, 130-134 (1972).
- Ladner, J. E., Finch, J. T., Klug, A., and Clark, B. F. C., *J. molec. Biol.*, **72**, 99-101 (1972).
- Suddath, F. L., Quigley, G. J., McPherson, A., Sneden, D., Kim, J. J., Kim, S. H., and Rich, A., *Nature*, **248**, 20-24 (1974).
- Marsh, D. J., and Petsko, G. A., *J. Appl. Cryst.*, **6**, 76-80 (1973).
- Richards, F. M., *J. molec. Biol.*, **37**, 225-230 (1968).
- Rubin, J., Brennan, T., and Sundaralingam, M., *Biochemistry*, **11**, 3112-3128 (1972).
- Kim, S. H., Berman, H. M., Seeman, N. C., and Newton, M. D., *Acta Cryst.*, **B29**, 703-710 (1973).
- Fresco, J. R., Adams, A., Ascione, R., Henley, D., and Lindahl, T., *Cold Spring Harb. Symp. quant. Biol.*, **31**, 527-537 (1966).
- Levitt, M., in *Polymerisation in Biological Systems* (edit. by Wolstenholme, G. E. W., and O'Connor, M.), 147-171 (Elsevier, Amsterdam, 1972).
- Arnott, S., *Progress in Biophysics and Molecular Biology*, **21** (edit. by Butler, J. A. V., and Noble, D.), 265-319 (Pergamon Press, Oxford, 1970).
- Rich, A., Davies, D. R., Crick, F. H. C., and Watson, J. D., *J. molec. Biol.*, **3**, 71-86 (1961).
- Hoogsteen, K., *Acta Cryst.*, **12**, 822-823 (1959).
- Yaniv, M., Favre, A., and Barrell, B. G., *Nature*, **223**, 1331-1333 (1969).
- Levitt, M., *Nature*, **224**, 759-763 (1969).
- Brown, D. M., in *Basic Principles in Nucleic Acid Chemistry* (edit. by Ts'O, P. O. P.), **2**, chapter 1 (Academic Press, London, 1974).
- Fuller, W., and Hodgson, A., *Nature*, **215**, 817-821 (1967).
- Levitt, M., *J. molec. Biol.*, **80**, 255-263 (1973).
- Woese, C., *Nature*, **226**, 817-820 (1970).
- Hill, C. W., Combriato, G., Steinhart, W., Riddle, D. L., and Carbon, J., *J. biol. Chem.*, **248**, 4252-4262 (1973).
- Holley, R. W., Apgar, J., Everett, G. A., Madison, J. T., Marquisee, M., Merrill, S. H., Penswick, J. R., and Zamir, A., *Science*, **147**, 1462-1465 (1965).
- Barrell, B. G., and Clark, B. F. C., *Handbook of Nucleic Acid Sequences* (Joynson-Bravvvers, Oxford, 1974).
- Schevitz, R. W., Navia, M. A., Bantz, D. A., Cornick, G., Rosa, J. J., Rosa, M. D. H., and Sigler, P. B., *Science*, **177**, 429-431 (1972).
- Kim, S. H., Quigley, G., Suddath, F. L., McPherson, A., Sneden, D., Kim, J. I., Weinzierl, J., Blattmann, P., and Rich, A., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 3746-3750 (1972).
- Klug, A., Robertus, J. D., Ladner, J. E., Brown, R. S., Finch, J. T., *Proc. natn. Acad. Sci. U.S.A.* (in the press).
- Robertus, J. D., Ladner, J. E., Finch, J. T., Rhodes, D., Brown, R. S., Clark, B. F. C., and Klug, A., *Nucleic Acids Res.*, **1**, 927-932 (1974).

letters to nature

A model of the magnetospheric substorm

It is generally recognised that the Earth's magnetic tail and the plasma sheet play a vital role in the processes responsible for the observational phenomenon known as the magnetospheric substorm. Many different models¹⁻⁵ of substorm behaviour have been described with differing degrees of success, and to propose another requires some justification. A new model, if it is to be useful, must do two things. It must be specific enough to make quantitative theoretical analysis possible, and it must fit the morphological features more completely or in a more elegant way than other models. In addition, it should be distinctive enough from other models to be separated from them by observational tests. I believe the model presented here has those properties.

The main features of the model are that the plasma sheet is formed on closed field lines and moves inward during the growth phase; the magnetic field line reconnection across the neutral sheet occurs on the lobe field lines, is essentially continuous but is not directly responsible for the expansion phase; and the expansion phase is the result of an interchange type of instability with the plasma sheet expanding on to empty, closed field lines.

The two principal regions in the tail—the plasma sheet and the lobes of the tail connected to the polar caps—are easily distinguished by their particle populations. The plasma sheet is denser and hotter than the lobe plasma⁶ and its energy density is of the same order as its magnetic energy density. The boundary between the two is normally not more than $1 R_E$ thick^{7,8}. One possible reason for the well defined boundary is that there is a source mechanism which only injects particles on plasma sheet field lines⁵. Alternatively, lobe field lines can be open allowing

plasma to escape down the tail, with the plasma sheet trapped on closed field lines⁹. I shall discuss the consequences of the latter.

A convenient starting point for the model substorm sequence is illustrated in Fig. 1a. The plasma sheet occupies all the closed field lines and at its end there is a neutral sheet which extends out of the plane of the paper on both sides. Suppose that the plasma flows towards the neutral sheet under the influence of an electric field (Fig. 1b). The open magnetic field lines of the tail would be reconnected at the neutral sheet at a steady rate. On the Earthward side of the neutral sheet the electric field would convect the plasma inward and accelerate the particles¹⁰. On the far side of the neutral sheet the plasma would be accelerated away from the Earth and gets lost down the tail. The important feature is that lobe field lines thus become reconnected and form closed field lines outside the plasma sheet (Fig. 1b). The plasma sheet, which contains relatively hot, dense plasma, becomes surrounded by closed field lines almost devoid of plasma in comparison. The magnetic field strength outside the plasma sheet would have to be stronger than inside in order to maintain a pressure balance across the boundary. The inward convection would continue (forming the growth phase of the substorm) until the plasma sheet boundary became unstable to a form of interchange instability. As a result the plasma would expand outwards to fill all the closed ex-lobe field lines and the magnetic field strength inside the plasma sheet would increase by a compressional wave propagating inwards from the position of the boundary. This, of course, is the expansion phase of the substorm (Fig. 1c). It leaves a region of turbulent plasma which would eventually thermalise during the recovery phase (Fig. 1d), returning the plasma to the starting point. The inward convection would continue during the recovery phase and may generate another substorm before the original sequence is complete.

The most important magnetospheric feature in this model is the outer boundary of the plasma sheet which initially convects inward stably and later becomes grossly unstable. There are plausible reasons why this should occur. If the model is to make any observational sense the quiet auroral arcs of the growth phase must connect to this boundary. Thus, there is high conductivity strip through the ionosphere on the field lines that pass through the boundary. That helps to stabilise the boundary by short circuiting the electric field of the developing instability. The expansion phase is triggered by the current, which is aligned with the field, becoming too large for the plasma to carry in a stable manner¹¹.

The outer boundary of the plasma sheet is likely to have a complex structure during any magnetospheric convection. The convective velocity, $\vec{V} = -(\vec{E} \times \vec{B})/B^2$, normal to the boundary must be continuous; therefore, because B changes, there must also be a change in the electric field as the boundary is crossed. The simple fluid concept of convective flow is quite inadequate to analyse that situation. It is necessary to take into account the three-dimensional nature of the boundary, currents aligned with the field, and the possibility of electric fields parallel to the magnetic field. It is thus not yet possible to project the electric field discontinuity at the boundary of the plasma sheet in the equatorial region down the field lines to the ionosphere to see whether it matches the electric field reversal region observed by low altitude satellites¹². A detailed theoretical analysis of the boundary is of crucial importance.

The source of the electric field must be considered. The field-

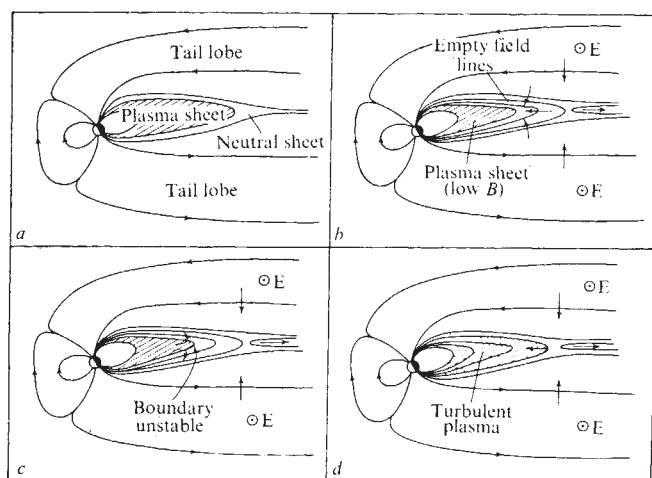


Fig. 1 *a* Basic configuration of the magnetosphere; *b*, the growth phase of the substorm, showing inward convection and reconnection; *c*, the expansion phase, caused by an interchange instability at the outer boundary of the plasma sheet; *d*, the recovery phase during which the turbulent plasma gradually thermalises. Arrows indicate plasma flow and the field strength in the lobes is not intended to be represented by the density of field lines; E , electric field, directed perpendicular to the plane of the page.

line reconnection takes place on lobe field lines and so the following equation, derived by Alfvén¹³⁻¹⁵ on the basis of self consistency between fields and currents, should be at least approximately true. It relates the total electric potential ϕ across the tail, to the field strength B_x and particle density N .

$$\phi = B_x^2 / 4\pi Ne$$

Thus the crosstail electric field is determined by the field strength, B_x , in the tail. B_x is controlled by the total magnetic flux in the tail and the configuration of the magnetopause¹⁹. There should be a direct relationship between energy input to the magnetosphere in the form of substorm activity and the magnetic field strength in the tail¹⁶.

The model obviously satisfies many of the gross morphological features of the magnetospheric substorm. The inward movement of the plasma sheet and the motion of quiet auroral arcs towards the equator are natural features. The outward movement of the plasma sheet and the inward magnetic field compression wave during the substorm expansion phase are directly related through the interchange nature of the instability. The poleward expansion of the aurora during breakup is also explained. It can easily accommodate such features as a multiple substorm onset¹⁷ because the boundary could become unstable over a range of longitudes. There is one auroral feature which does not seem to fit easily into the model—the multiple arc system. It has been reported¹⁸ that the most vigorous breakups occur when they are initiated on the arc nearest to the equator, and not on that furthest from the equator, as may be expected in the model.

There is one morphological feature of this model which differentiates it from most other models¹⁻⁵. If the model is correct it should be possible to cross the neutral sheet, or the region where the magnetic field along the tail changes sign, without detecting the plasma sheet before the onset of a substorm.

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Lunar electrical conductivity

THE lunar magnetometer experiment¹ has made important contributions to studies of the lunar interior. The magnetic fluctuation data can be used to estimate the structure of lunar electrical conductivity because it allows comparisons of predicted and observed lunar electromagnetic responses. There are many complications in the correct computation of predicted responses, and approximations are always used. There has been a gradual evolution of models, each improvement including more and more complications, with resultant changes in the estimated conductivities. The first models² had a solar wind incident on the Moon from all sides, in order to approximate the front side lunar response. The backside data was modelled by a Moon in a vacuum¹. Improvements in the front side response were made by incorporating the effects of a finite solar wind velocity³. Previous calculations on a cylindrical model⁴ indicated that the presence of the void region behind the Moon could also modify the sunlit side response. Formal solutions to the electromagnetic problem in such a geometry have been presented⁵.

We have carried out numerical inversions of the lunar electromagnetic response, incorporating in the calculations a void region behind the Moon. We used two different numerical methods. They both gave similar results, although one method is definitely superior in its ability to satisfy the boundary conditions near the limb of the void region. This method consists of using a series of electric and magnetic multipoles, situated at the centre of the Moon, in order to express the field in the void region, which is caused by currents and charges inside the Moon and on its sunlit surface. By using dual Fourier-Bessel series⁶, we can associate to each multipole a series of spherical TE and TM modes that satisfy the boundary conditions on the surface of the cylinder. The amplitude of each multipole is then determined by applying the boundary conditions on the lunar surface.

The inclusion of the void region behind the Moon substantially lessens the predicted lunar response on the sunlit

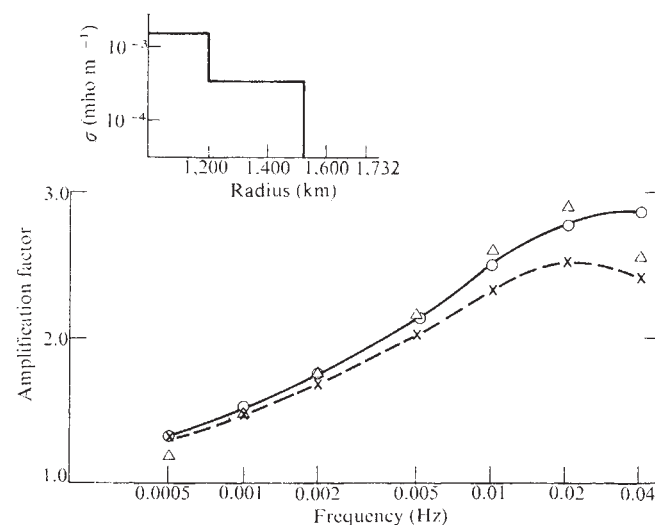


Fig. 1 The theoretical amplitude of the transfer function obtained by considering the void region behind the moon (x), and that obtained by assuming the void to be filled with plasma (O). Δ , Data values.

side, especially at shorter periods (Fig. 1). This effect occurs because some of the internal field is escaping out into the void region. In order to build up the response again, conductivities are required near the surface. Figure 2 shows a model which gives a good fit to the observed amplification on the front side and which also agrees well with the backside

- ¹ Russell, C. T., and McPherron R. L., *Space Sci. Rev.*, **15**, 250 (1973).
- ² Axford, W. I., Petschek, H. E., and Siscoe, G. L., *J. geophys. Res.*, **70**, 1231 (1965).
- ³ Atkinson, G., *J. geophys. Res.*, **71**, 5157 (1966).
- ⁴ Parks, G. K., Laval, G., and Pellat, R., *Planet. Space Sci.*, **20**, 1391 (1972).
- ⁵ Hill, T. W., and Dessler, A. J., *Planet. Space Sci.*, **19**, 1275 (1971).
- ⁶ Akasofu, S.-I., Hones, E. W., Bame, S. J., Asbridge, J. R., and Lui, A. T. Y., *J. geophys. Res.*, **78**, 7257 (1973).
- ⁷ Hones, E. W., Asbridge, J. R., and Bame, S. J., *J. geophys. Res.*, **76**, 4402 (1971).
- ⁸ Buck, R. M., West, H. I., and D'Arcy, R. G., *J. geophys. Res.*, **78**, 3103 (1973).
- ⁹ Axford, W. I., *Rev. Geophys.*, **7**, 421 (1969).
- ¹⁰ Axford, W. I., and Hines, C. O., *Can. J. Phys.*, **39**, 1433 (1961).
- ¹¹ Swift, D. W., *Planet. Space Sci.*, **15**, 1225 (1967).
- ¹² Swift, D. W., and Gurnett, D. A., *J. geophys. Res.*, **78**, 7306 (1973).
- ¹³ Cowley, S. W. H., *Cosmic Electrodynamics*, **3**, 448 (1973).
- ¹⁴ Bornatici, M., and Schindler, K., *J. geophys. Res.*, **79**, 529 (1974).
- ¹⁵ Alfvén, H., *J. geophys. Res.*, **73**, 4379 (1968).
- ¹⁶ Fairfield, D. H., *Planet. Space Sci.*, **20**, 1455 (1972).
- ¹⁷ McPherron, R. L., Russell, C. T., and Aubry, M. P., *J. geophys. Res.*, **78**, 3131 (1973).
- ¹⁸ Akasofu, S.-I., *Polar and magnetospheric substorms* (Reidel, Dordrecht, 1968).
- ¹⁹ Coroniti, F. V., and Kennel, C. F., *J. geophys. Res.*, **77**, 3361 (1972).

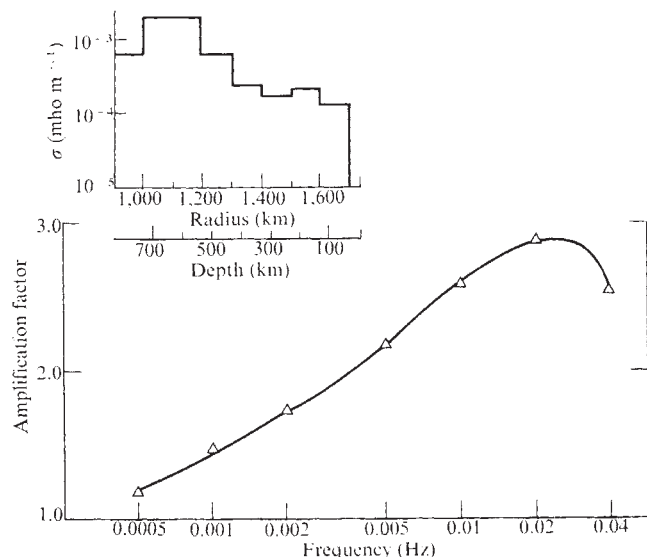


Fig. 2 Amplitude of the transfer function of an 8 layer model, calculated by including the void region behind the moon.

response⁷. There is a remarkable similarity between the conductivity values in the top 200 km of the Moon and those in the Earth's crust⁸. The temperature and pressure conditions in these regions of the Moon are probably quite similar to terrestrial crustal conditions, but in the Earth's crust the conductivity is probably associated with the presence of free water in the rocks. This possibility seems most unlikely for the Moon. A water-saturated lunar model, in fact, does not give a good fit to the electromagnetic response data, as it does not provide the increase conductivity-depth relationship that is needed because of the decrease of porosity with pressure. If free water is not present, then a semiconduction type of mechanism is required in order to explain the degree of conductivity observed at low temperatures.

In order to examine this more closely, various models of lunar temperature were assumed, and the electromagnetic response data were inverted to find the parameter of a semi-

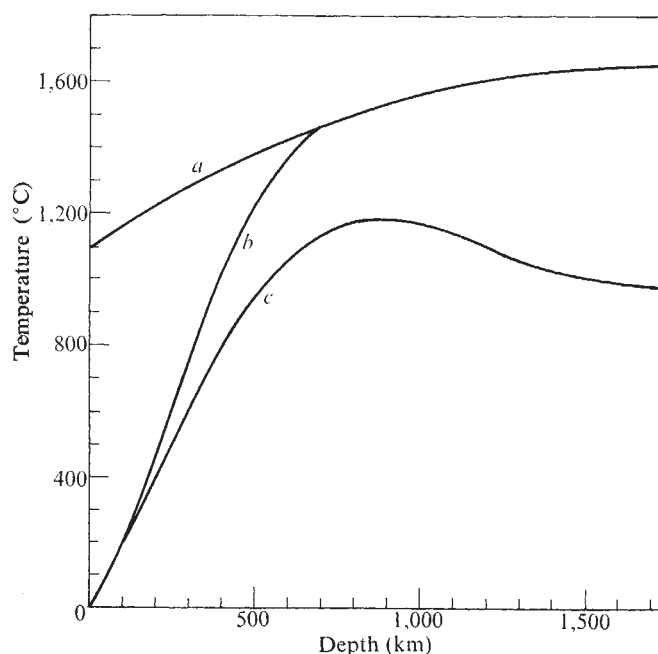


Fig. 3 Model of the temperature distribution inside the moon. *a*, Solidus (anhydrous basalt); *b*, uranium concentration = $2.3 \times 10^{-8} \text{ g g}^{-1}$; *c*, $U = 1.1 \times 10^{-8} \text{ g g}^{-1}$.

conductor characterised by a conductivity prefactor, σ_0 , and activation energy, E_0 . The temperature models used are those of Toksöz *et al.*⁹ (Fig. 3). A very good fit to the data was obtained using this two-parameter model (Fig. 4). The parameters were not very sensitive to the temperature models (Table 1).

Table 1 Parameters of lunar semiconductor model

High temperature model	Low temperature model
$\sigma_0 \text{ mho m}^{-1} \ 2.1 \times 10^{-3}$	3.1×10^{-3}
$E_0 \text{ eV} \ 0.13$	0.14

This low activation energy results from the fact that the electromagnetic data can accommodate only a moderate increase in conductivity at depths characterised by a sharp thermal gradient. Figure 5 shows a comparison of the conductivity values with laboratory measurements of terrestrial and lunar rocks. Low activation energy conduction has been observed in lunar rocks but the conductivity level is much lower than that inferred for the Moon¹⁰. These experiments were, however, plagued by thermal hysteresis. This was subsequently attributed to the experimental procedure¹¹. We are not yet able to draw definite conclusions on the lunar composition.

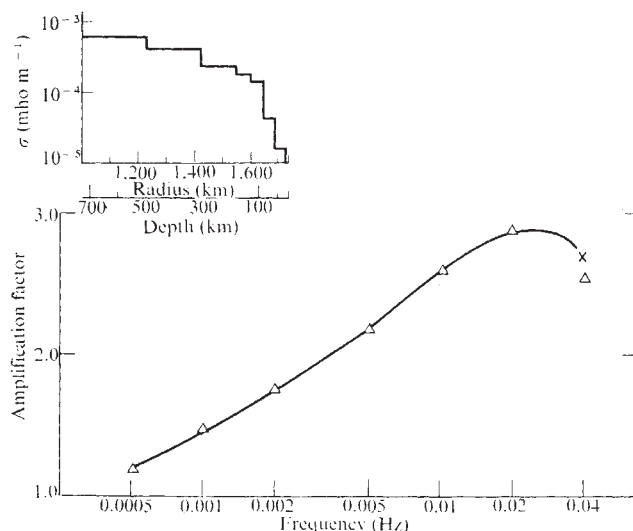


Fig. 4 Amplitude of the transfer function of a semiconductor lunar model which satisfies the high temperature curve in Fig. 3. The model values, *x*, agree with the data, Δ , except at 0.04 Hz

There is very little resolution in the present electromagnetic data for the electrical properties at depths greater than 700 km, and, therefore, another conduction mechanism cannot be identified. The shallower conductivity parameters were so insensitive to the temperature model variations, that temperature inferences could not be made from these results.

The model results were calculated by assuming the lunar surface magnetometer to be at the subsolar point, and the source field to propagate parallel to the downstream cylindrical cavity. The magnetic amplification depends, however, on detailed considerations of the location of the sensor relative to the subsolar point, and on the orientation of the magnetic field of the solar wind. Moreover, the data are sensitive to certain parameters of the solar wind. The results shown in Table 1 and Fig. 5 were computed using a solar wind velocity of 300 km s^{-1} . If 400 km s^{-1} is used, the conductivity parameters increase by 50% in σ_0 , and by 25% in E_0 . In this case the conductivity value of $10^{-4} \ \Omega^{-1} \text{ m}^{-1}$ is not reached until a depth of 150 km. The fluctuation of plasma pressure of the solar wind, which accompanies the magnetic fluctuations, is another parameter that can influence the results. An empirical correc-

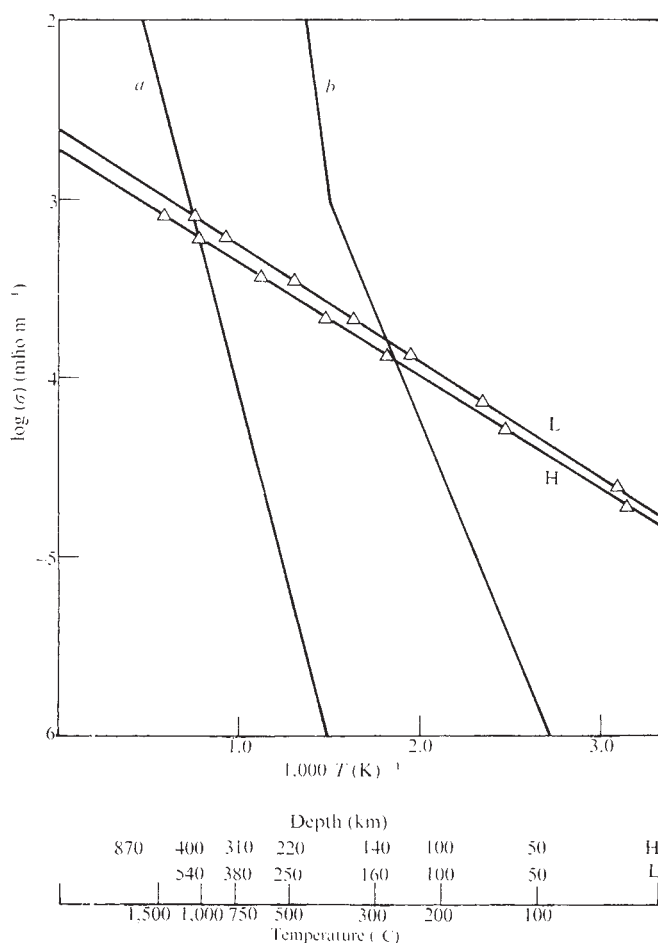


Fig. 5 Log σ against $10^{-3} T^{-1}$ for a semiconductor lunar model with the high (H), and low (L) temperature distribution shown in Fig. 3. Also shown are the laboratory data for *a*, terrestrial olivine sample (10.4% fayalite); *b*, lunar basalt sample 10024.22.).

tion for the interaction of the plasma with the remanent field at the Apollo 12 site has already been applied to the data³ and the resulting set of data agrees fairly well with that obtained at the Apollo 15 site¹². We have not included in our calculation the possible effect that these fluctuations might have on the diamagnetic signature usually observed in the downstream cylindrical cavity. We do not know to what extent these parameters can be reconstructed from the original observations.

These factors render our conclusion somewhat tentative but we believe, nevertheless, that the results suggest rather strongly that a conduction mechanism with a low activation energy plays an important role in determining the conductivity at shallow and moderate depths inside the Moon.

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- ⁵ Schwartz, K., and Schubert, G., *J. geophys. Res.*, **78**, 6496 (1973).
- ⁶ Smythe, W. R., *Static and Dynamic Electricity*, third ed., 211 (1968).
- ⁷ Schubert, G., Smith, B. F., Sonett, C. P., Colburn, D. S., and Schwartz, K., *J. geophys. Res.*, **78**, 3688 (1973).
- ⁸ Brace, W. F., *Geophys. Monogr.*, **14**, 243 (1971).
- ⁹ Toksoz, M. N., Solomon, S. C., Minear, J. W., and Johnston, D. H., *The Moon*, **4**, 190 (1972).
- ¹⁰ Schwerer, F. C., Nagata, T., Fisher, R. M., *The Moon*, **2**, 408 (1971).
- ¹¹ Schwerer, F. C., Huffman, G. P., Fisher, R. M., and Nagata, T., *Fourth Lunar Sci. Conf.*, 663 (1973).
- ¹² Smith, B. F., Schubert, G., Colburn, D. S., Sonett, C. P., and Schwartz, K., *Fourth Lunar Sci. Conf.*, 683 (1973).

Tungus event was not caused by a black hole

JACKSON and Ryan¹ suggested that a black hole may have caused the Tunguska catastrophe in 1908, in which an explosion occurred in a remote part of Siberia. About 10^{23} erg (equivalent to 2 Mton of TNT) of energy was released. Several lines of evidence render the black hole hypothesis extremely unlikely.

The explosion blew down trees in open places within a radius of 30–40 km and seared them within a radius of 15–18 km. The exposed roots were directed towards the centre of the area². In ravines, partially protected trees remained standing, but many had had their tops broken.

These and other characteristics of the event indicated that the main part of the energy went into an explosion in the air. By contrast, a typical, solid nickel-iron meteorite first buries itself below the surface and then dissipates its energy in a subsurface explosion. The Barringer crater in Arizona, which is 200 m deep, and 1 km in diameter, was produced in this way. No significant excavation was caused by the Tunguska explosion.

It has been suggested that the event was caused by the impact of a small cometary nucleus, consisting of a mass of frozen gases mixed in with nickel-iron and silicate particles^{2–4}. The whole body would then have had a low degree of cohesion, so that it would have fragmented in the air and dissipated most of its kinetic energy before it reached the surface.

The air blast could also have resulted from the impact of a small black hole with a diameter of the order of angstroms and an asteroidal mass. The black hole would, however, have passed through the Earth in 10–15 min and caused a similar explosion at the point of exit, which would have occurred in the North Atlantic between 30°–40° W and 40°–50° N (ref. 1).

Microbarographs in the London and Cambridge areas recorded infrasound from the explosion³. The waves arrived at the centroid of the stations—51°30'N, 0°2'W—at approximately 0515 UT, on June 30, 1908. The impact itself occurred at 60°55'N, 101°57'E at approximately at 0017 UT (ref. 2). The approximate great circle distance from the point of impact to the centroid of the stations is 5,720 km, so the average speed of the waves was about 320 m s⁻¹, which is about the usual value for this type of wave.

It is clear that the recorded waves were travelling from Siberia, and not from the North Atlantic, because the microbarograph at Cambridge recorded the wave 10 min or so before the time that it was recorded at Petersfield. Infrasound waves from the site of the suggested exit explosion should however, have arrived in the London area between about 0215 UT and 0330 UT—about three hours before the arrival of the Siberian wave—assuming that any waves from either source travelled with the same velocity. We have examined copies of the English microbarograph records, but have been unable to find any sign of waves from the suggested exit explosion.

In the London area, the maximum recorded amplitude

¹ Dyal, P., and Parkin, C. W., *Proc. second Lunar Sci. Conf.*, **3**, 2391 (1971).

² Sill, W. R., and Blank, J. L., *J. geophys. Res.*, **75**, 201 (1970).

³ Sonett, C. P., Smith, B. F., Colburn, D. S., Schubert, G., and Schwartz, K., *Proc. third Lunar Sci. Conf.*, **3**, 2309 (1972).

⁴ Reisz, A. C., Paul, D. L., and Madden, T. R., *The Moon*, **4**, 134 (1972).

of the Siberian waves was about 150 μ bar. It is possible to resolve amplitudes which are as low as 20–30 μ bar, so that any waves not observed must have had amplitudes which were less than about 20% of those of the Siberian waves. The suggested exit site is, however, closer to London than is the Tunguska region, so the recorded amplitudes of exit waves at London should have been greater, than those of the waves from Siberia in the absence of unusual propagation effects. We have considered whether strong zonal winds to the west could have caused ducting effects which reduced the amplitude of the eastward travelling exit wave to below 20–30 μ bar. Ducting between the mesosphere and the Earth's surface reduces greatly the amplitudes of waves with periods shorter than a few tens of seconds when they are travelling upwind, but it reduces the amplitudes of waves with periods of 1–2 min, such as those recorded from the explosion, by only a factor of two or three. If such ducting had been in effect, any waves from the exit explosion should still have had amplitudes of about 100 μ bar in the London area. In addition, the eastward travelling reverse wave from the explosion was observed on a Fues barograph at the Potsdam Geophysical Observatory about 26 h after the direct waves². If the eastward wave from the entrance explosion could propagate against the zonal winds it seems unlikely that eastward waves from an exit explosion could not do so as well.

Other arguments against the black hole hypothesis have been given by Wick and Isaacs⁵. The spatial pattern of numerous small magnetic globules with high nickel content found in the Tunguska region indicates that the source of these extraterrestrial particles was the impact explosion. Eyewitness accounts noted a thick dust train along the path of the fireball immediately after its passage, which is consistent with the deposition of material in the atmosphere, rather than loss of air into a black hole. The first night after the fall was exceptionally bright everywhere in Europe and western Siberia. In the Caucasus it was possible to read a newspaper at midnight without artificial light. That implies deposition of extraterrestrial material in the upper atmosphere simultaneously with the impact. The wide area of atmospheric deposition is comparable to the dimensions of a cometary tail, and is not compatible with the idea of slow transport of dust vertically and horizontally from a ground level explosion. The deposition in the upper atmosphere of both fine cometary dust and icy material could give rise, at high latitudes and at that time of year, to a phenomenon like noctilucent clouds. That could account for the bright nights.

Fessenkov⁶ investigated the measurements of the transparency of the atmosphere made in California in 1908, and found that from approximately the middle of July to the second half of August 1908, a noticeable decrease in the coefficient of transparency occurred. Fessenkov concluded that it was caused by the dissipation in the atmosphere of material from the Tunguska object. He estimated the total mass of dissipated material to be several million tons.

All the evidence favours the idea that the impact which caused the Tunguska catastrophe involved a body with characteristics like a cometary nucleus, rather than a black hole.

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- ¹ Jackson, A. A., and Ryan, M. P., *Nature*, **245**, 88 (1973).
- ² Krinov, E. L., in *The Moon Meteorites and Comets* (edit. by Middlehurst, B. M., and Kuiper, G. P.), (Univ. Chicago Press, Chicago, 1963).
- ³ Whipple, F. J. W., *Q. Jl R. Met. Soc.*, **16**, 287 (1930).
- ⁴ Astapovich, I. S., *Soviet Astron.*, **10**, 465 (1933).
- ⁵ Wick, G. L., and Isaacs, J. D., *Nature*, **247**, 139 (1974).
- ⁶ Fessenkov, V. G., *Meteoritika*, **6**, 8 (1949).

The formation of the Earth

THREE recent hypotheses for the origin of the inner planets are based on chemical data obtained from meteorites or from solar observation^{1–5}. The chondritic earth model of Ringwood¹ or the cold accretion model (see summary in ref. 2) require partial melting of meteorite material and segregation of metal and sulphide to form a core, but the fractionation must have been a rapid, disequilibrium process to leave 2,000 p.p.m. Ni in the silicate mantle. The heterogeneous accretion model^{3,4} overcomes the problem of high Ni in the mantle by having accretion of the metal core first, followed by silicate mantle and finally by volatile-bearing material which formed the crust, no two fractions having been in equilibrium. Lewis⁵ maintains that condensation from a homogeneous nebula under differing physical conditions can account for the density variation between the inner planets without invoking the metal-silicate fractionation suggested by Urey⁶. Compared to the above hypotheses based on meteorite composition, the following approach is more direct as it is based on a reliable estimate of modern upper mantle composition.

The best estimate of undepleted upper mantle composition obtained from a study of some 200 spinel-lherzolite xenoliths in basalt, is as follows: SiO₂ 45.0, TiO₂ 0.09, Al₂O₃ 3.5, Cr₂O₃ 0.41, total Fe as FeO 8.0, MnO 0.11, NiO 0.25, MgO 39.0, CaO 3.25, Na₂O 0.28, K₂O 0.035; sum 99.92 (weight). (R. H., A. L. Chambers, D. K. Paul, and P. G. Harris, unpublished). This composition is close to that of three undepleted garnet-lherzolite xenoliths from the Bultfontein kimberlite pipe, but it has much lower TiO₂, Na₂O and K₂O contents than Ringwood's pyrolite¹.

In meteorites, Ni is always concentrated in the metal relative to the silicate. For example, the metal of pallasites has about 12% Ni (weight), but coexisting olivine has only some 20 p.p.m.⁷. If an Fe–Ni metal fraction had been segregated from silicate mantle to form or to enlarge the core, then Ni should have been almost quantitatively removed from the upper mantle. The Ni:Fe ratio of modern upper mantle, about 0.03, is much lower than that in meteorites but higher than that of the silicate of meteorites. One possibility put forward here is that Ni was almost totally removed from the mantle during early segregation of metal and sulphide into the core, then partially replenished by the addition of further meteorite material. If this assumption is made, the composition of pre-replenishment, Ni-free upper mantle can be calculated by subtracting a suitable amount of Ni carrier from modern upper mantle.

The most Ni-rich carrier, ataxitic iron meteorite, would require about 0.15 lunar mass to be mixed into the Ni-free upper mantle and oxidised. In addition to providing the 2,000 p.p.m. Ni, about 1% FeO (weight) would also have been introduced. At the other extreme, the maximum mass of material capable of providing the necessary amount of Ni would be just under three lunar masses of type 1 carbonaceous chondrite (C1). C1 fragments have been identified in a variety of meteorite types^{8,9}. In addition, C1 material has probably been incorporated into lunar regolith^{10,11}, and so seems a better candidate as Ni carrier than the rare ataxites. Although other types of chondrite are also potential Ni carriers, some C1 is necessary to provide the earth's volatiles and so the model presented here will consider C1 only.

Table 1 Calculation of lithophile element content of primitive Ni-free mantle

Atomic	*	†	‡	§	
Si	10,000	10,000	2,000	8,000	10,000
Ti	118	15	5	10	12.5
Al	923	915	174	741	926
Cr	75	72	25	47	59
Mg	12,359	12,900	2,100	10,800	13,500
Ca	731	773	140	633	791

* 'Pyrolite'¹

† Modern upper mantle from composition given in text

‡ 20% carbonaceous chondrite, from ref. 12

§ † minus ‡

|| §, recalculated to 10,000 Si atoms – primitive Ni-free (and Na-free) upper mantle

To allow comparison between the upper mantle and meteorite groups, the method used for calculating the composition of hypothetical, Ni-free upper mantle is that of Larimer and Anders¹². In Table 1 the lithophile element contents of 'pyrolite'¹ and upper mantle as given above are expressed in atoms per 10,000 Si atoms. By subtracting 20% C1 from the modern upper mantle composition and recalculating to 10,000 Si atoms, primitive, Ni-free upper mantle composition is obtained. This has a 100 FeO:(FeO + MgO) molecular ratio of 5. In Table 2, the data of Table 1 are expressed relative to carbonaceous chondrite composition; ordinary and enstatite (E) chondrite compositions are also included.

Table 2 Lithophile element fractionation relative to carbonaceous chondrites

	*	†	‡	§		¶
Si	1.0	1.0	1.0	1.0	1.0	1.0
Ti	0.46	0.55	4.3	1.0	0.74	0.55
Al	1.06	1.05	1.06	1.0	0.71	0.55
Cr	0.47	0.58	0.59	1.0	0.82	0.77
Mg	1.29	1.23	1.18	1.0	0.90	0.74
Ca	1.13	1.10	1.04	1.0	0.67	0.53

* Recalculated Ni-free primitive upper mantle, from Table 1

† Modern upper mantle from composition given in text

‡ 'Pyrolite'¹

§ Carbonaceous chondrite

|| Ordinary chondrite¹²¶ Enstatite chondrite¹²

From Table 2 it is evident that fractionation of the lithophile elements has been different on Earth from that in meteorites. The sequence carbonaceous to ordinary to E chondrites has fairly regular depletion in all five elements, whereas in the upper mantle (column †) Al, Mg and Ca are enriched relative to carbonaceous chondrites, but Ti and Cr are depleted. 'Pyrolite'¹ shows the depletion in Cr, but not in Ti; this is probably due to a failing in Ringwood's model. The recalculated, Ni-free upper mantle composition shows the same trends as modern upper mantle, but accentuated by the subtraction of unfractionated material. Lack of coherence within the lithophile element group requires a mechanism different from any responsible for fractionation between the different chondrite groups, as does enrichment in Mg over Al and Ca. Gain by the Earth of the Mg, Ca, Al-rich fraction preferentially lost by ordinary and E chondrites¹² would have resulted in an impoverishment in Mg over Al and Ca in the upper mantle. These two problems are dealt with separately below.

Depletion of the upper mantle in Ti and Cr is best explained by their partial removal into the core as sulphides. In the highly reduced E chondrites, virtually all Cr occurs as the sulphide daubreélite¹³, and 75% of the Ti is present in troilite¹⁴. The figures in Table 2, column*, indicate that, in the upper mantle, conditions were less strongly reducing than those which obtained during the formation of the E chondrites, for, in the

former, a much higher proportion of the Ti and Cr remained as oxides. This is borne out by the presence of Fe in the upper mantle, there being virtually no oxidised Fe in E chondrites. Reducing conditions on Earth probably were not strong enough for reduction of significant SiO₂ to the metal, which occurred to limited extent in E chondrites. It is therefore unlikely that the light element in the Earth's core is Si. Removal of over half the Ti and Cr from the upper mantle probably took place during the early differentiation which also removed the Ni. The depletion in Ti and Cr and enrichment in Al, Mg and Ca cannot be accounted for by heterogeneous accretion.

Mg enrichment over Al and Ca could be the result of the Earth having gained pure forsterite (Mg₂SiO₄). I think, however, that the following two possibilities are more probable. Each necessitates SiO₂ loss from the upper mantle. The first and perhaps simpler explanation is that the mantle differentiated into upper and lower portions, with preferential enrichment of the latter in FeO and SiO₂ (refs 2, 15). This would be acceptable to geophysicists, whose models for lower mantle composition range from pyroxenite to harzburgite with molecular 100 FeO:(FeO + MgO) ratios from 36 to 20 (ref. 2). But this mechanism requires greater stability in the lower mantle of Ca and Al relative to Mg, a property which seems difficult to explain. The second alternative is that loss of almost three lunar masses of SiO₂ by evaporation from the upper mantle could have produced the observed Mg, Al and Ca enrichment, if primitive mantle had ordinary chondritic Mg/Si, Al/Si and Ca/Si ratios. The 35% SiO₂ loss necessary would have been accompanied by total depletion in alkalis and volatiles, but the addition of 20% C1 Ni carrier would have replenished the upper mantle in these; if the Na content of C1 of Schmitt *et al.*¹⁶ is used, the 0.28% Na₂O in the upper mantle is exactly accounted for. Abundances of other trace elements and alkalis in the upper mantle are not sufficiently well known for rigorous testing of this hypothesis. This second alternative is attractive in that an early period of surface heating might have radically altered the pattern of convection in the earth to ultimately bring about the distinction between upper and lower mantle. The second alternative could also apply to a homogeneous mantle, but in this case over ten lunar masses of SiO₂ would have had to have been lost.

The chemical composition of the upper mantle is incompatible with a single model based on heterogeneous accretion or cold accretion. Geophysical evidence for lower mantle composition² combined with the upper mantle composition given above suggest that the bulk mantle has a 100 FeO:(FeO + MgO) molecular ratio of about 20, in the range of the ordinary chondrites. Ordinary chondrite parent is also required to explain the enrichment in the upper mantle of Mg over Al and Ca. Although Anders¹⁷ maintains that the five components of meteorites (lithophile elements, metal, silicate, sulphide and volatiles) should each be considered independently in modelling planetary composition, the evidence put forward here argues that this might not be necessary.

My preferred model for the formation of the Earth is as follows: accretion began either as a metal-rich core followed by ordinary chondrite material, or as iron-rich ordinary chondrite-like material alone. Partial melting resulted in loss of siderophile and chalcophile elements from the silicate mantle to the core. Surface heating by a superluminous sun drove off volatiles, alkalis and about 35% of the SiO₂ from the upper part of the primitive mantle. Addition of C1 material during protracted cooling of the earth added Ni, alkalis, and, finally, volatiles. That accretion took place on an extended time scale is evidenced by meteorites, some of which have grains irradiated by solar flares in their interiors; such irradiation must have occurred after the dissipation of the bulk of the nebular gas¹⁸.

Along the midplane, pressure in the solar nebula increased towards the centre¹⁹. The series carbonaceous to ordinary to E chondrites represents material accreted under increasing

nebular gas pressure²⁰⁻²². Because the oxidation state of the Earth is close to that of ordinary chondrites, the more highly reduced E chondrites probably originated within the orbit of the Earth. This possibility is currently being considered.

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- ¹ Ringwood, A. E., *Geochim. cosmochim. Acta.*, **30**, 41-104, (1966).
- ² Anderson, D. L., Sammis, C., and Jordan, T., *Science*, **171**, 1103-1112 (1971).
- ³ Turekian, K. K., and Clark, S. P., jun., *Earth planet. Sci. Lett.*, **6**, 346-348 (1969).
- ⁴ Grossman, L., *Geochim. cosmochim. Acta*, **36**, 597-619 (1972).
- ⁵ Lewis, J. S., *Earth planet. Sci. Lett.*, **15**, 286-290 (1972).
- ⁶ Urey, H. C., *Geochim. cosmochim. Acta.*, **1**, 209-277 (1951).
- ⁷ Buseck, P. R., and Goldstein, J. I., *Science*, **159**, 300-302 (1968).
- ⁸ Fodor, R. V., and Keil, K., *Abs. in Meteoritics*, **8**, 366-367 (1973).
- ⁹ Wilkening, L. L., *Geochim. cosmochim. Acta*, **37**, 1985-1989 (1973).
- ¹⁰ Baedeker, P. A., Chou, C.-L., Sundberg, L. L., and Wasson, J. T., *Earth planet. Sci. Lett.*, **17**, 79-83 (1972).
- ¹¹ Krähenbuhl, U., Ganapathy, R., Morgan, J. W., and Anders, E., *Geochim. cosmochim. Acta, Suppl.*, **4**, 1325-1348 (1973).
- ¹² Larimer, J. W., and Anders, E., *Geochim. cosmochim. Acta*, **34**, 367-388 (1970).
- ¹³ Mason, B., *Geochim. cosmochim. Acta*, **30**, 23-39 (1966).
- ¹⁴ Keil, K., *J. geophys. Res.*, **73**, 6945-6976 (1968).
- ¹⁵ Kumazawa, M., Sawamoto, H., Ohtani, E., and Masaki, K., *Nature*, **247**, 356-358 (1974).
- ¹⁶ Schmitt, R. A., Goles, G. G., Smith, R. H., and Osborn, T. W., *Meteoritics*, **7**, 131-213 (1972).
- ¹⁷ Anders, E., *A Rev. Astron. Astrophys.*, **9**, 1-34 (1971).
- ¹⁸ Pellas, P., Poupeau, G., Lorin, J. C., Reeves, H., and Audouze, J., *Nature*, **223**, 272-274 (1969).
- ¹⁹ Cameron, A. G. W., and Pine, M. R., *Icarus*, **18**, 377-406 (1973).
- ²⁰ Larimer, J. W., and Anders, E., *Geochim. cosmochim. Acta*, **31**, 1239-1270 (1967).
- ²¹ Blander, M., *Geochim. cosmochim. Acta*, **34**, 61-76 (1971).
- ²² Larimer, J. W., *Geochim. cosmochim. Acta*, **37**, 1603-1623 (1973).

Needham and Francheteau² showed that, in the area visited by Archimède, steep, inward facing slopes of the deepest outward tilting blocks of the Rift Valley walls bound an uneven, regionally flattish Inner Floor. The central part of this is occupied by a ridge, 200 m high and asymmetric along at least part of its length. The ridge, or Central High², which we have named Mont de Vénus, is about 4 km long and 1 km wide. Two marginal topographic highs, or plateaus, mapped during the course of a detailed narrow beam echo-sounding survey made by the French naval vessel d'Entrecasteaux, lie along the bottom of the bordering scarps of the Inner Floor (cv. Schrumph, cc. Caillaud, and V. Renard, personal communication). The marginal highs extend further south than Mont de Vénus and may be more rectilinear in gross shape.

The width between the major bordering scarps of the Inner Floor is 3.3 km. Assuming a mean opening rate of about 2.2 cm yr⁻¹, the Inner Floor may be entirely younger than 1.5 × 10⁵ yr, an inference supported by the occurrence here of very fresh lavas². All seven dives of Archimède took place within the Inner Floor, and all were concentrated in an area of less than 5 km² near 36° 50' N (Fig. 2).

The major purpose of the dives was to show that useful, detailed geological mapping of very rough deep sea terrain, such as the Inner Floor, can be undertaken from a manned submersible. It was further hoped that new evidence could be gathered, which would help to answer two important questions. First, is the surface expression of the boundary between the two diverging lithospheric plates confined to one of the highs or deeps within the Inner Floor, or is the locus of emplacement of new crust spread over a wider zone? And second, is the structural pattern of the Inner Floor steady state or transient? With these problems in mind, an effort was made to search for evidence of tectonic activity and to establish specific associations between microtopography and lava morphologies. A close, precisely controlled look was taken at a considerable part of Mont de Vénus, and at the eastern deep and adjacent marginal high (Fig. 2).

About 9 km was covered by the dives, in visual contact with the ocean floor. The position of the Archimède was known within 10-200 m, depending on her distance from the nearest of the acoustic transponders that were moored to the bottom and used for navigation. The transponder network for positioning Archimède was tied, with an accuracy of about 50 m, to the networks used by the d'Entrecasteaux in the making of the detailed surface-ship map that was used as a base for the dives. The scale of features that could be resolved (0-20 m) is clearly beyond the reach of surface-ship, or even deep-towed, instrumentation. A downward looking sonar and a pressure gauge were used to measure the altitude and relative depth of Archimède respectively. These were recorded every 10 s on digital printouts, and the precision was about 1 m. Useful complementary information was provided by a panoramic sonar device with a maximum range of 1,400 m. A dual system of still and television cameras (which kept film and videotape recordings) gave good images of the sea floor, whether it was flattish or very steep; the usual distance of observation was 3-5 m, and the maximum distance about 10-15 m. A telemanipulated grab was used for sampling and four pillow fragments, together with small quantities of sediment, were collected during the course of the dives.

Mont de Vénus is largely covered by recent lava flows. It is a lenticular structure with apparent dextral offsets. Above a water depth of 2,560 m its summit is less than about 500 m wide and deepens progressively northwards (Fig. 2). The surface of the summit is characterised by large (approximately 1 m) globular (dome like) lava forms (Fig. 3a) partially immersed in pale, carbonate-rich sediment. The globular structures may represent the surface ex-

Inner floor of the Rift Valley: first submersible study

THE Rift Valley of the Mid-Atlantic Ridge¹, within which lies a segment of the accreting plate boundary between Africa and North America, is well defined between 36° 40' N and 36° 55' N. It is about 30 km wide and 1.5 km deep in that area (Fig. 1). This small portion of the rift, WSW of the Azores, was chosen as the primary target of the French-American Mid-Oceanic Undersea Survey (FAMOUS) programme, and many surface ship studies have been conducted already. We report here preliminary results of seven dives into the deepest part of the Rift Valley that were made by the bathyscaphe Archimède during the summer of 1973.

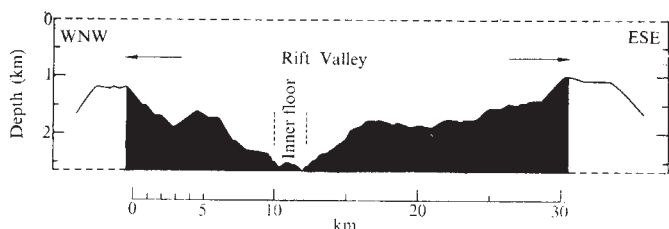


Fig. 1 Topographic cross section of the Rift Valley near 36° 50' N, based on surface-ship data², showing the setting of the Inner Floor in the area of the Archimède dives (Fig. 2).

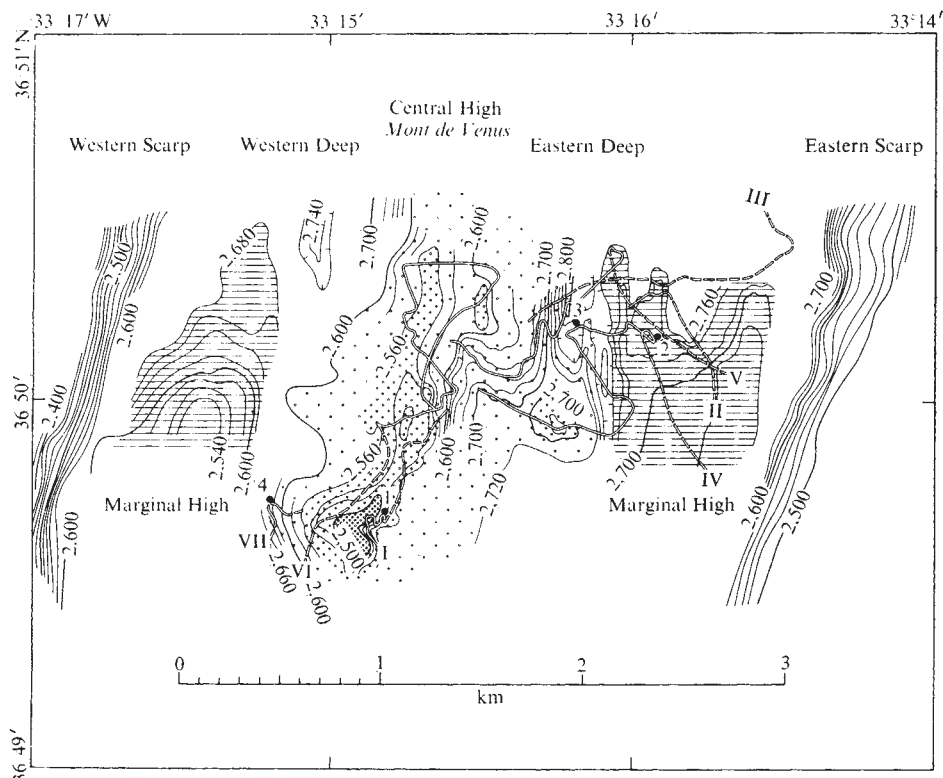


Fig. 2 Map of the Inner Floor of the Rift Valley of the Mid-Atlantic Ridge (Fig. 1), showing tracks of Archimède and positions of rock samples taken from Archimède. Roman numerals, sequences of dives 1–7; ●, rock samples. Different ornamentation for Central High and marginal highs helps to distinguish these features. The map is based on Archimède data along tracks and other contours are sketched from the d'Entrecasteaux map. Depths shown are from pressure gauge and downward looking sonar measurements made from Archimède, or are normalised to this base; corrected depths recorded by precision echo-sounders from surface ships are approximately 30 m smaller. The average cruising speed of the bathyscaphe was less than 1 knot when in visual contact with seafloor. The map is tied to Europe 50 coordinates. Depths are in metres. Track of Archimède: continuous double lines, visual contact with ocean floor; broken double lines, sonar contact with ocean floor.

pression of the top of a gently dipping, coherent lava flow unit (J. G. Moore, personal communication). The sediment may be thin, but it occupies up to 80% of the fields of view from the bathyscaphe. No large open fissures, nor any definite indications of small fissures, were seen. On the crest of the summit, relatively gentle slopes are distributed over a width of 50 m, and the organisation of lava flows shows that the crest coincides with the dividing line between eastern and western sets of flows, both directed downslope. In addition, mapping from Archimède demonstrated that the steep south-eastern slopes of the summit of Mont de Vénus are lobate in character. The wavelength of the lobes was typically 50–100 m.

The topography on the eastern side of Mont de Vénus down to the bordering deep is of a staircase type, with precipitous, north–south scarps, free of sediment, separating relatively flattish areas where the sediment cover is sporadic. One of the scarps is about 100 m high and has a slope of about 80° (Fig. 2). There is also a prominent east–west trending slope near 36° 50'N (Fig. 2). Cylindrical lava forms several metres long, which we call bolsters (Fig. 3b), are present on all of the traversed scarps. The bolsters are commonly associated with shorter, phallus shaped forms which we call phalli (Fig. 3b), and with lava forms resembling entrails which we call tripe (Fig. 3c). Some of the lavas associated with the scarps suggest regular stacking. Tali of lava fragments (Fig. 3d) occur typically at the feet of the scarps; and domed or globular, and commonly striated forms, similar to those seen on the summit, are a feature of the flatter areas between scarps. Collapsed or broken pillows (Fig. 3e) and bolsters occur on the scarps and on the flatter areas.

The short section of slope crossed by the bathyscaphe on the western side of Mont de Vénus has a relatively uniform gradient and is less steep than scarps crossed on the eastern side, but it is similarly typified by bolsters which were directed downslope. Some of these reach lengths of as much as 7 m, and many collapsed forms are present. No fields of angular boulders were seen, and sediment cover of up to 15–20% was observed.

The scarps bordering the deep to the east of Mont de

Vénus follow north–south directions rather than the dominant NE–SW trend of the summit. Isolated boulders lie on a blanket of sediment on the flattish floor of the deep, and some of them are obviously detached from adjacent flow fronts. The floor of the deep is represented, at least locally, by an extremely narrow (15–20 m) corridor (Fig. 2).

East of the deep, a series of ridges, with a relief of about 50 m or more, represent northward deepening fingers of the marginal high that lies between Mont de Vénus and the major eastern scarp of the Inner Floor. The topographic grain of the eastern region seems to be more linear than that near the summit of Mont de Vénus. Slopes of the eastern ridges, however, are similarly covered by coherent lavas, including elongated forms which are directed downslope; the measured flow directions are predominantly north–west to west on the westward facing slopes, and mainly east on the eastward facing slopes. Sediment cover, locally prominent in the flatter areas, is sparse on the slopes which represent the inner edge of the eastern marginal high. There is appealing local evidence, along these slopes, of a north–south, possibly normal, fault with a 2–3 m throw (Fig. 3d).

The four pillow fragments collected by Archimède come from very well known positions (Fig. 2) and, although not sampled from outcrops, they are evidently derived from adjacent flows. The rocks vary in size from 20–40 cm in diameter, and weight 15–47 kg. They are well jointed, have glassy selvages in various states of preservation and are similar, in mineralogy and chemistry, to other rocks from the Mid-Atlantic Rift Valley³.

Samples 1 and 4 from Mont de Vénus are olivine tholeiites with olivine crystals set in a matrix containing olivine, plagioclase and traces of spinel. Sample 2, an aphanitic pyroxene tholeiite from east of the eastern deep, is made up of equal amounts of plagioclase and clinopyroxene. Sample 3, a picritic basalt from the eastern depression itself, contains abundant, large olivine crystals set in a groundmass of plagioclase laths, olivine and a dark mesostasis; minor spinel is present both within the olivine, or as isolated crystals. The two Mont de Vénus tholeiites are more

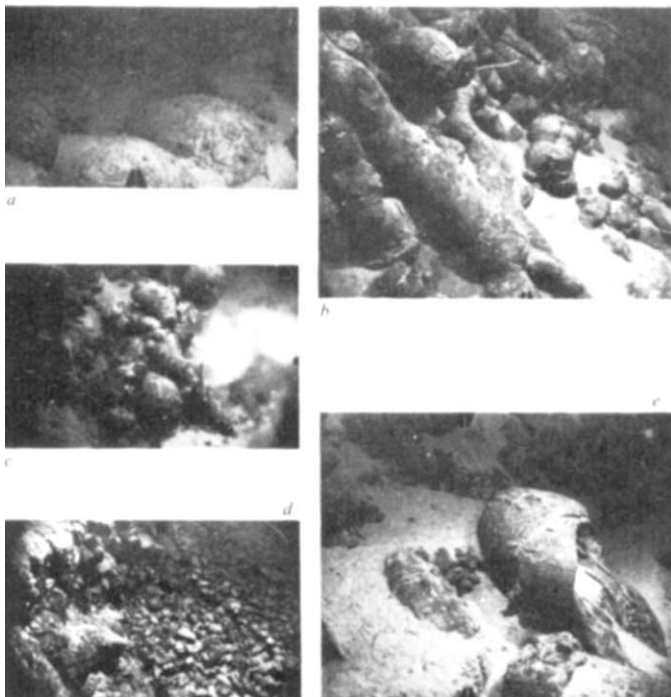


Fig. 3 Different types of lava morphologies on the Inner Floor of the Rift Valley, mapped from the bathyscaphe. *a*, Globules, partially immersed in sediment; particularly characteristic of flat and gently dipping areas. Photograph taken on summit of Central High. *b*, Bolsters and shorter blunt ended forms (phalli); directed downslope; typically found on several steep and very steep slopes where sediment cover is absent or sporadic; note cracked glassy selvages and longitudinal striae. Photograph taken on western slope of eastern marginal high. *c*, Tripe, frequently associated with, or occurring on, other lava forms found on slopes. Photograph taken on western part of eastern marginal high. *d*, Talus of well jointed lava fragments at foot of steep, probably faulted scarp; in general, sediment-free talus is typical of feet of steep slopes including those covered by lava forms which are directed downslope. Photograph taken on western slope of eastern marginal high. *e*, Pillow, broken egg structure and frozen lava flow; similar isolated lava forms lie commonly on less steep parts of slopes and on flattish areas where sediment cover is relatively abundant. Photograph taken on western edge of eastern marginal high.

mafic, with higher MgO (10%), and lower TiO₂ (0.8–0.9%) and K₂O (0.15–0.2%) content than the tholeiite sampled further east (6.4% MgO; 1.6% TiO₂; and 0.4% K₂O). The most mafic of all four sediments is the picritic basalt (sample 3) which contains 22.6% MgO, 0.5% TiO₂ and 0.06% K₂O (P. Cambon, personal communication).

The four specimens show different degrees of surface weathering. Using the thickness of manganese¹ and palagonite⁵ as indicators, and assuming a mean manganese accumulation rate of 3 μm per 10³ yr, suggests that all of the samples are younger than about 10⁵ yr. The youngest rocks are the two samples from Mont de Vénus; these are younger than 10⁴ yr. Sample 3 from the eastern depression is at least twice as old and sample 2 at least three times as old. The data thus suggest the possibility that the age of the Inner Floor increases eastwards from Mont de Vénus.

The results of the dives, taken together, lead to some general conclusions. Steep, and very steep (80–90°), major slopes of the Inner Floor near 36° 50'N are associated with bolsters which are in a downslope direction and other elongated and blunt ended lava forms. Lava talus is clearly associated with the feet of the scarps. Globule (dome) shaped, and commonly striated pillows lie more

or less buried in sediments on flattish areas between scarps. Incipient break-up of many of the intact lava forms is indicated by jointing and cracking.

The lobate structures and the distribution pattern of lava forms on scarps and slope surfaces indicate that Mont de Vénus is a constructional volcanic feature, with the corollary that its major scarps are the fronts of one or more flow units. It is, nevertheless, possible that flows which are directed downslope mask evidence of vertical movements that have contributed to the process which formed the scarp. Such an interpretation would be consistent with the steepness of the scarps and with the partially demonstrated bilateral asymmetry of Mont de Vénus. The apparently straighter trend of structures east of the eastern deep which flanks Mont de Vénus, and the local evidence of faulting along the inward facing slopes of the eastern deep, point rather more persuasively to some tectonic influence between the faulted scarps that border the Inner Floor. Indeed, the mapped part of the eastern deep near 36° 50'N may be a tensional fissure and the volcanic activity reflected by the downslope flows east of the eastern deep may be associated with dilation and small scale vertical movements.

The observation that the two rocks sampled from Mont de Vénus are younger than those sampled further east suggests, in a tentative way, that Mont de Vénus is the youngest part of the Inner Floor. The dominant, and approximately central position of Mont de Vénus within the Inner Floor adds some weight to this inference, as does the limited evidence that the relief of the marginal highs has been more subject to modification by vertical movements than that of Mont de Vénus itself. It seems, therefore, quite possible, although young lavas are found over a considerable part of the width of the Inner Floor, that Mont de Vénus represents the most recent surface expression of new crust along the plate boundary.

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¹ Heezen, B. C., Tharp, M., and Ewing, M., *Spec. Pap. geol. Soc. Am.*, **65**, 122 (1959).

² Needham, H. D., and Francheteau, J., *Earth planet. Sci. Lett.*, **22**, 29–43 (1974).

³ Muir, I. D., Tilley, C. E., and Scoon, J. S., *J. Petrology*, **7**, 193–201 (1966).

⁴ Bender, M. L., Ku, T.-L., and Broecker, W. S., *Science*, **151**, 325–328 (1966); *Earth planet. Sci. Lett.*, **8**, 143–148 (1970).

⁵ Moore, J. G., *Prof. Pap. US geol. Surv.*, **550**, 163–171 (1966).

Negative magnetic anomaly associated with Mount Kenya

MOUNT Kenya is a large central volcano just south of the equator in central Kenya. It rises to a height of 5,199 m above sea level. The base of the volcano covers an area of approximately 7,000 sq km, and is roughly circular in plan with an average diameter of 105 km. A detailed geological study¹ indicated that some 1,500 m of the upper part of the mountain has been removed by erosion since the volcano was formed in the Plio-Pleistocene.

The highest peaks of the mountain are formed from the eroded central plug which is about 2.2 km in diameter. There are a number of small glaciers in the peak area, the

intensity, associated with the peak area and displayed by all the observations.

All field measurements were made with a proton precession magnetometer and have been reduced to a common epoch time of 1430 UT on March 16, 1974, by using the continuous variometer records from the Nairobi geomagnetic observatory. The correlations for epoch were small and less than 60 γ in all cases ($1\gamma \equiv 10^{-8}$ T). In addition to the field measurements on the volcanic plug, measurements were taken at six sites along the main approach track from the township of Naro Moru which is situated 32 km due west of the peak of Mount Kenya. (Fig. 1 and Table 1).

Corrected values of total intensity, F , have been plotted as a function of the distance of each measuring site from

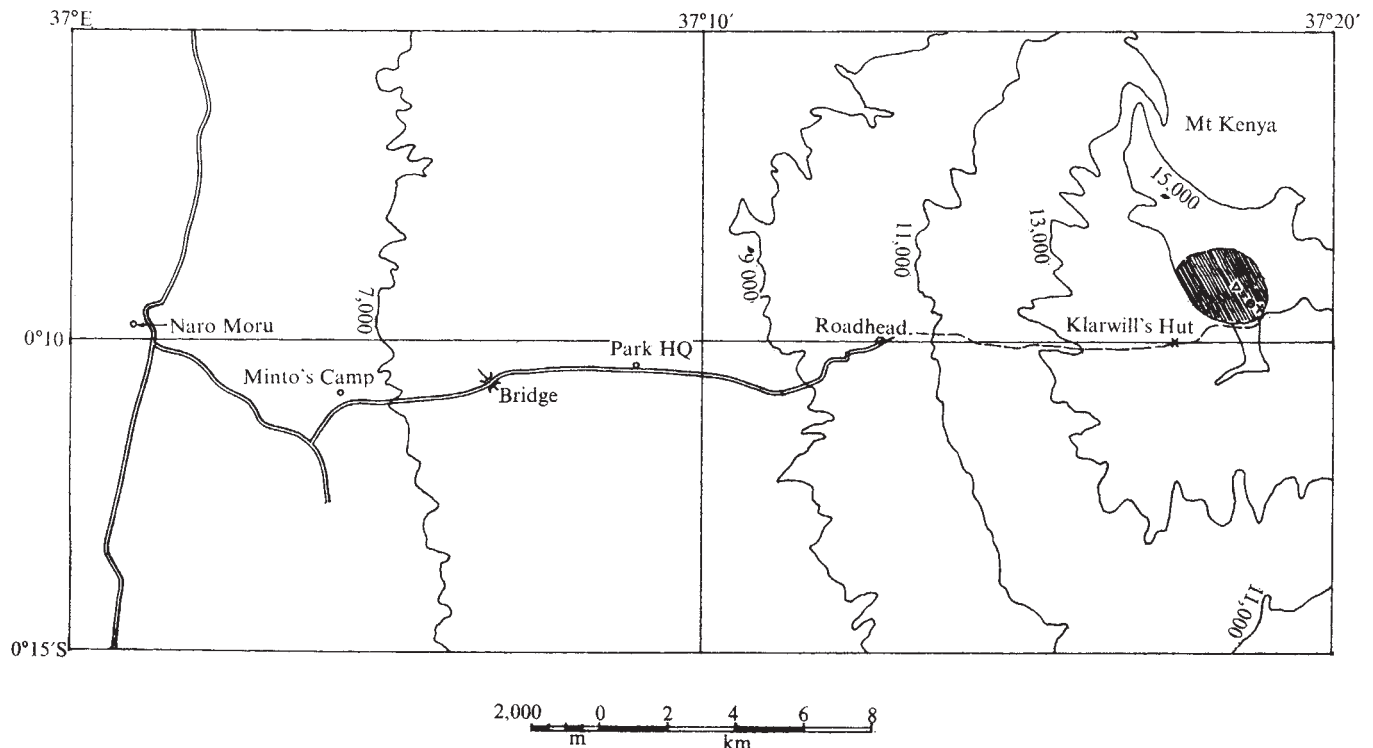


Fig. 1 Sketch map of the positions of the measuring sites along the track from Naro Moru to Batian. Contours are in feet. Shaded area, volcanic plug; $\odot$, Lewis Glacier; Δ , Batian; $\times$, Top Hut.

largest of which, the Lewis glacier, is being studied in detail by one of us (S.H.) As part of this study, with a view to determining the thickness of the ice and the shape of the underlying topography, close-spaced measurements were taken of the total intensity of the geomagnetic field along two profiles across the Lewis glacier at altitudes near 4,785 m. The glaciological interpretation of these observations will be reported in detail elsewhere. We here draw attention to a large negative anomaly in the total field

Batian, the highest peak of Mount Kenya (Fig. 2). The expected regional field value for the area (34,440 γ) obtained from a recent magnetic survey of the whole of Kenya (N.J.S.) is also shown (Fig. 2). A shallow negative anomaly of up to -400 γ exists over the whole traverse (all of which is underlain by volcanics from Mount Kenya), and the anomaly is sharply accentuated to greater than -1,300 γ on the volcanic plug. A somewhat similar negative anomaly of even larger amplitude has been reported on the volcano

Table 1 Data from the measurement sites

Map No.	Measuring site	Latitude	Longitude	Altitude (m)	Distance from Batian (km)	Total field F , corrected to common epoch (γ)
1	Naro Moru	0°09.7'S	37°00.9'E	1,975	32.5	34,283
2	Minto's Camp	0°10.8'S	37°04.3'E	2,100	26.4	34,283
3	Bridge (Naro Moru River)	0°10.9'S	37°06.5'E	2,195	22.4	34,334
4	Near Park HQ	0°10.4'S	37°09.0'E	2,440	17.7	33,957
5	Roadhead	0°10.1'S	37°12.8'E	3,050	11.0	34,073
6	Klarwill's Hut	0°10.0'S	37°17.5'E	4,145	2.5	34,057
7	Top Hut	0°09.6'S	37°18.9'E	4,785	1.2	33,118
8	Lewis Glacier (1)	0°09.5'S	37°18.8'E	4,725	0.7	33,610
9	Lewis Glacier (2)				0.9	< 33,350

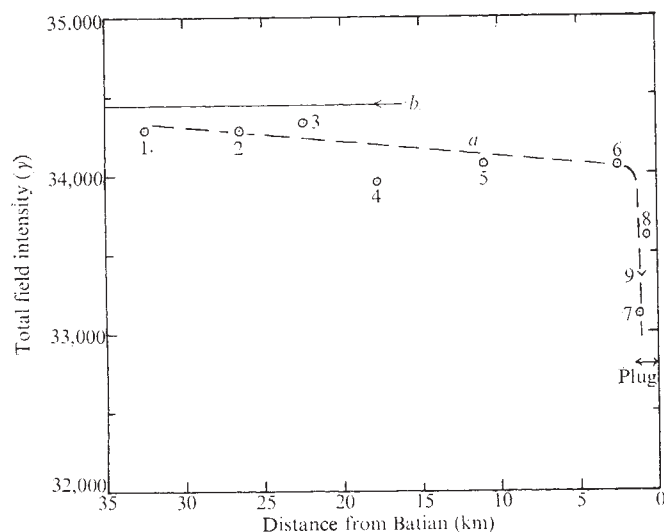


Fig. 2 a, Total field intensity as a function of distance from Batian. (Table 1 gives a key to the numbered sites); b, expected regional field.

Kilimanjaro in northern Tanzania², and in this case also the anomaly increases sharply near the peak (Kibo) of the mountain.

The plug of Mount Kenya is composed of a central core of nepheline syenite with outer partial sheaths of fine grained phonolite which in places is penetrated and locally altered by the syenite. Baker¹ considers that the main inner syenite body is younger than the adjacent phonolite but it is not clear whether all the syenite was emplaced in a single phase of volcanic activity. Evernden and Curtis³ have given isotopic ages of 2.64 Myr for a specimen of nepheline syenite taken from the plug and of 3.1 Myr for phonolite from the main eruptive phase collected near Embu, 40 km south-east of the peaks. The intense local negative field anomaly associated with the plug suggests that the rock of which it is composed possesses strong 'normal' remanent magnetisation, that is, it was emplaced at a time when the Earth's magnetic field was in the same direction as at present. Furthermore, an oriented specimen of phonolite collected at the edge of the Lewis glacier, near Top Hut, was strongly magnetised in a 'normal' sense. The present magnetic field measurements and the isotopic date of 2.64 Myr for the syenite suggest that the last eruptive phase through the main vent of Mount Kenya occurred during the Gauss normal epoch. The high degree of erosion of the peak area certainly precludes eruption during the present Brunhes epoch. On the other hand, for Kilimanjaro, the relatively slight degree of erosion of the Kibo crater, and the large negative magnetic anomaly², point to a much younger main eruptive phase within the Brunhes normal epoch, and this is confirmed by the isotopic ages in the range 0.365–0.514 Myr for basalts from Kilimanjaro³.

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¹ Baker, B. H., *Rep. geol. Surv. Kenya*, **79** (1967).

² Emeleus, T. G., and Osborne, D. G., *Ann. Géophys.*, **24**, 715–718 (1968).

³ Evernden, J. F., and Curtis, G. H., *Curr. Anthropol.*, **6**, 343–384 (1965).

⁴ Cox, A., *Science*, **163**, 237–245 (1969).

The sub-Palaeozoic basement in central Ireland

THERE is no direct evidence to indicate the nature of the sub-Palaeozoic basement beneath central Ireland. The recognition of gneissic xenoliths in Viséan volcanics in this region is therefore significant. They have been located at Croghan Hill in County Offaly (National grid ref. N4833; Fig. 1), Clare Castle (N2447), and the Dungolman River (N1851) in County Westmeath.

At the first locality one specimen, 30 cm³ in volume, was found in agglomerates cropping out to the west of Croghan village, and a second specimen of 1,600 cm³ in basalt a little further south. From agglomerates near Clare Castle 17 specimens were obtained, ranging from 10–200 cm³. The agglomerates from this latter locality occur as loose

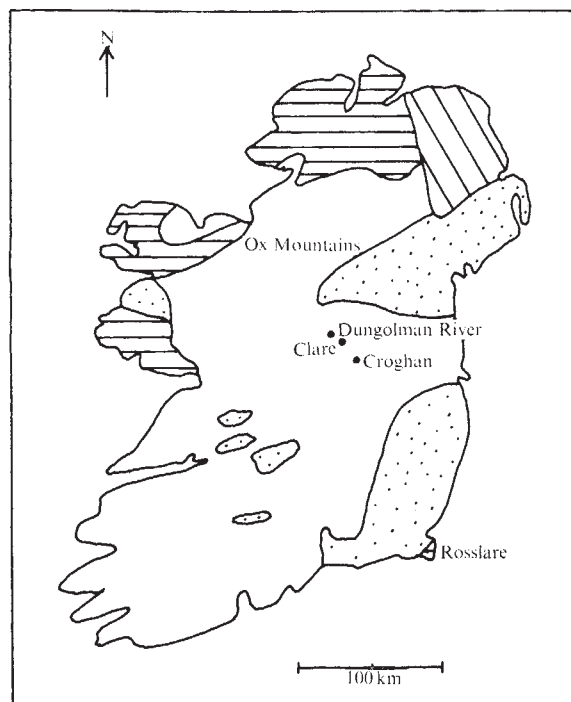


Fig. 1 Localities referred to in the text and areas of Dalradian or older rocks (horizontal ruling), Lower Palaeozoic rocks (shading), Upper Palaeozoic rocks (blank) and Mesozoic and Tertiary rocks (diagonal ruling).

boulders, often of great size, and they are certainly of local origin. They predominate in the boulder train and their lithic content, of Upper Palaeozoic age, closely matches that from beneath the area, which is known from bore-holes (K. Cullen, personal communication). Similar agglomerates outcrop 11 km to the north-west in the bed of the Dungolman River. Material dredged from the bed of the river includes basalt fragments containing a further 10 xenoliths ranging from 10–30 cm³.

The Clare Castle gneisses are coarse grained and weakly banded, and contain the mineral assemblage: garnet-sillimanite-potash feldspar-plagioclase-quartz-rutile. Euhedral garnet, up to 2 mm across, has a composition close to the pyrope-almandine join at about 56% almandine. Sillimanite prisms up to 10 mm long define a strong lineation. Both feldspars are quite fresh. The plagioclase composition is An_{45–50}, and it is unzoned; the potash feldspar is a string perthite. Modes vary within the range: garnet 20–30%, sillimanite 5–20%, plagioclase 25–30%, potash feldspar 10–20% and quartz 10–40%. Other phases occur only in small amounts. Books of graphite up to 2 mm long occur in most specimens and there are traces of phlogopite in a

few cases. The assemblage belongs to a high amphibolite or granulite facies.

The gneiss from the agglomerate at Croghan is very similar, though it lacks potash feldspar. That from the basalt is very altered; only coarse sillimanite and quartz remain in a matrix of secondary chlorite, fibrous amphibole and altered glass. Abundant pseudomorphs after garnet can be seen readily, however, and Watts¹ has observed garnet in xenoliths from the area.

The gneisses from the Dungalman River are undoubtedly of granulite grade: they contain the critical assemblage hypersthene-garnet. They are rich in basic plagioclase (about An₆₀), hypersthene, garnet and quartz, and are medium grained and weakly banded. There is some alteration to anthophyllite, chlorite and carbonates.

Other lithic fragments are abundant in the agglomerates. Many compare with phyllites and slates of currently accepted Ordovician age which are exposed in the surrounding Lower Palaeozoic massifs. These rocks are of an extremely low metamorphic grade. That is in strong contrast to the gneisses which are therefore likely to be Dalradian or older. Indeed, the only rocks of similar type in Ireland are pre-Dalradian. The Dalradian everywhere differs in lithology²⁻⁴ and is generally of much lower grade, though it reaches middle amphibolite facies in places. The older Deer Park Schists⁴ are also unlike the xenoliths. Gneissic rocks from north-western Mayo⁵ and the Rosslare Complex^{6,7}, which may be Lewisian, are mica bearing and poor in garnet, and of a distinctly lower grade. The only comparable rocks are those from the Precambrian of the Ox Mountains, which Lemon⁸ has tentatively referred to the Moianian. These differ in that they contain kyanite.

The gneisses are clearly much older than the Lower Palaeozoic sediments. It is most unlikely that the gneiss has been derived indirectly, from the Old Red Sandstone (ORS) for example. None of the 29 xenoliths has adhering sediment, and gneissic clasts do not occur in the ORS exposed in nearby areas, or in ORS blocks within the agglomerates themselves. The gneiss must have been derived from the sub-Palaeozoic basement.

That is of interest in view of recently proposed plate tectonic models of Precambrian and Lower Palaeozoic history. Most of those require oceanic crust to lie beneath the Southern Uplands and its structural continuation into central Ireland⁹⁻¹³. Though some are not clear on the point, others clearly show this to be present even after the final Caledonian deformation. Powell¹⁴ has pointed out that the geophysical evidence conflicts with that idea, and proposed that there is a sialic basement "of Lewisian-type rocks" beneath the Southern Uplands. Jeans¹⁵ has accepted this, and his model shows oceanic crust lying, virtually pinched out, beneath the Midland Valley. The evidence presented here strongly support the latter hypotheses.

It is improbable that the gneiss was derived from a detached slice of sialic crust thrust over the oceanic basement. The localities discussed here are separated by 35 km, and the furthest is 70 km from Strokestown, the nearest point on the northern subduction zone proposed by Dewey^{6,7}. It is far more likely that beneath this area there is a true basement of high grade metamorphic rocks. It seems probable that this basement extends beneath the Down-Longford Massif and into the Southern Uplands.

I would like to thank Mr K. Cullen of Irish Base Metals Ltd, who brought my attention to the Clare agglomerates, and IBM who supplied drilling logs from the area. I also thank Drs J. R. Andrews and P. S. Kennan for discussion and criticism.

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- ¹ Watts, W. W., *Guide to the collection of rocks and fossils of the Geological Survey of Ireland* (H.M.S.O. Dublin 1895).
- ² Cambray, F. W., *Am. Ass. Petrol. Geol., Memoir 12*, 181 (1969).
- ³ Hartley, J. J., *Proc. R. Ir. Acad.*, **41**, 218 (1933).
- ⁴ Phillips, W. E. A., Kennedy, M. J., and Dunlop, G. M., *Am. Ass. Petrol. Geol., Memoir 12*, 194 (1969).
- ⁵ Sutton, J. S., and Max, M. D., *Geol. Mag.*, **106**, 284 (1969).
- ⁶ Baker, J. W., *Proc. R. Ir. Acad.*, **69**, 1 (1970).
- ⁷ Max, M. D., and Dhonau, N. B., *Scient. Proc. R. Dubl. Soc.*, **4**, 103 (1971).
- ⁸ Lemon, G. G., *Geol. Mag.*, **108**, 193 (1971).
- ⁹ Dewey, J. F., *Nature*, **222**, 124 (1969).
- ¹⁰ Dewey, J. F., *Scott. J. Geol.*, **7**, 219 (1971).
- ¹¹ Bird, J. M., Dewey, J. F., and Kidd, W. S. F., *Nature*, **231**, 28 (1971).
- ¹² Fitton, J. G., and Hughes, D. J., *Earth planet. Sci. Lett.*, **8**, 223 (1970).
- ¹³ Garson, M. S., and Plant, J., *Nature phys. Sci.*, **242**, 34 (1973).
- ¹⁴ Powell, D. W., *Scott. J. Geol.*, **7**, 369 (1971).
- ¹⁵ Jeans, P. J. F., *Nature phys. Sci.*, **245**, 120 (1973).

Reversals of the Earth's magnetic field and climatic changes

It has been suggested that evolution may be influenced by reversals of the Earth's magnetic field¹⁻³. It was observed that there were correlations between discontinuities in microfossil assemblages (or evolutionary discontinuities) and reversals of the Earth's magnetic field in deep sea sediment cores⁴⁻⁶. This observation was put on a firm base when Hays⁷ showed statistically that reversals directly or indirectly exert a selective influence on radiolaria. The explanation offered by Uffen^{1,2} and Simpson³ for the connection between evolution and reversals was that during reversals of the Earth's magnetic field, the intensity of the field would be reduced to a very low value, allowing organisms at the surface of the Earth to be bombarded by increased cosmic radiation normally shielded by the field. This increased radiation should then cause an enhanced mutation rate and hence produce an evolutionary discontinuity. It was, however, shown later that the estimated increase in the mutation rate would be small and unlikely to cause evolutionary discontinuities⁸⁻¹⁰.

Two other ways have been suggested in which reversals of the Earth's magnetic field could influence evolution. One way is by the direct biological effect of a magnetic field on organisms; there is a short discussion of this by Crain¹¹. The second mechanism proposes that a reversal of the Earth's magnetic field could cause a change in the climate of the Earth and hence indirectly produce faunal extinctions¹⁰.

Here, we shall discuss the second proposed mechanism and speculate on a possible causal relationship between reversals and climate. Evidence has been previously presented (for example refs 12, 13) suggesting a connection between the Earth's magnetic field and climate. Wollin *et al.*¹² showed that the record of mean annual temperature (averaged over 10-yr periods) was inversely correlated with the magnetic field intensity at nearby magnetic observatories. They were, however, unable to suggest what the causal relationship between these two parameters was. King¹³ has discussed the possibility that the Earth's field controls in some unknown way the variations in pressure to be found at high latitudes in the troposphere. King has also suggested¹⁴ that solar ionising particles are responsible for some of the correlations to be seen between the yearly mean sunspot number and various meteorological phenomena.

Roberts and Olson¹⁵ have demonstrated a relationship between one climatic parameter and geomagnetic disturbances and have also offered an explanation for this relationship. They have

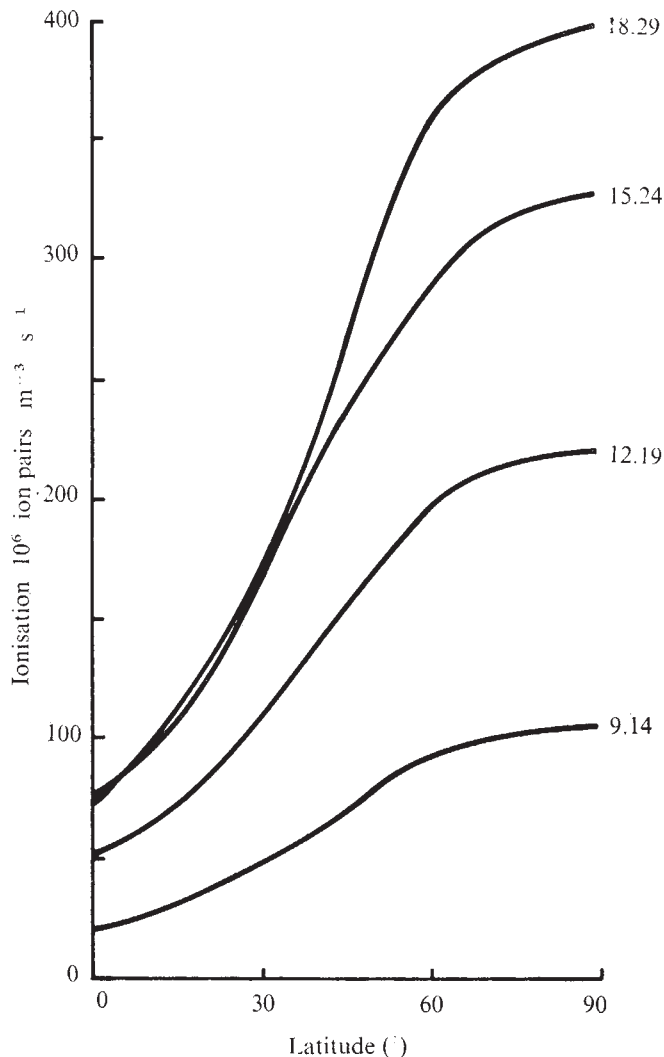


Fig. 1 Ionisation as a function of latitude. Curves are shown for four altitudes (measured in km).

shown¹⁵ that troughs at the 300 mbar level are influenced by geomagnetic disturbances. They found that, during the winter, troughs which moved into the Gulf of Alaska between 2 and 4 d after a geomagnetic storm underwent a greater degree of intensification than did troughs which appeared at the same place and during the same season but not within the 2–4-d time lapse after a geomagnetic storm. They suggest that this intensification may be caused by modification of the black body radiation from the relatively warm North Pacific as a result of increased cirrus cloud cover. It is thought by Roberts and Olson that increased ionisation at the tropopause level, caused by the ionising particles associated with the geomagnetic disturbances, could cause ion-induced nucleation and, hence, enhanced formation of clouds; the increased cirrus cloud cover at high latitudes during the winter results in a decrease in cooling rate which, in turn, leads to the increased vorticity.

A reversal of the Earth's magnetic field must be classified as a major geomagnetic disturbance. It is believed that the period of reduced field intensity during a reversal lasts between 1,000 and 10,000 yr^{16,17}. During the time of very low magnetic field intensity, the whole Earth would receive the present-day polar cosmic ray ionisation rate, all other factors being assumed to remain constant. We can estimate this rate by using the data of Saylor *et al.*¹⁸ for latitudinal variations in cosmic-ray ionisation rate as a function of altitude.

Table 1 gives ionisation rates at three different altitudes and at the equator and pole, taken from ref. 18. The top figure in each category gives the rate during a solar activity maximum, and the lower figure gives the rate during a solar activity minimum. Means

of the two values are given, and the polar/equatorial ratios of the means are shown in the last column.

Figure 1 shows the mean ionisation rate plotted as a function of latitude for four different altitudes. It can be seen that half or more of the Earth's area at these altitudes would have the ionisation rate increased by a factor of two or more during a reversal, if it is assumed that the polar ionisation rate is applicable to the whole Earth during a reversal. Using these curves and performing a simple numerical integration to determine average ionisation rates over the Earth's surface, it can be shown that the average ionisation increases by factors of 3.03 (for the lowest altitude curve), 2.87, 2.84 and 3.11 (for the highest altitude curve). If Roberts and Olson¹⁵ are correct in assuming that ionisation at such levels in the atmosphere is important for the production of cirrus clouds, then we should expect profound changes in the Earth's climate during a reversal, due to increased cloud cover at lower latitudes.

Table 1 Ionisation rate, (10^6 ion pairs $m^{-3} s^{-1}$) (measured at a pressure of 101.3 kN m^{-2})

Altitude (km)	Equator	Pole	Ratio
9.144	14.3 } 21.4 28.6 }	102.4 } 104.7 107.1 }	4.9
12.192	33.3 } 52.3 71.4 }	211.9 } 219.0 226.2 }	4.2
15.240	69.0 } 77.3 85.7 }	285.7 } 326.2 366.7 }	4.2

The specific mechanism which Roberts and Olson¹⁵ suggest as being responsible for the formation of cirrus, ion-induced nucleation, is highly speculative. Castleman¹⁹ presents evidence that ions can promote water vapour nucleation at supersaturation ratios considerably below that required for homogeneous nucleation; he suggests, for example, that at the summer mesopause (130 K, water concentration 10^8 cm^{-3}) sufficient supersaturation exists for this nucleation process to be operative. But Castleman does not state if this process would be significant lower in the atmosphere. Montefinale *et al.*²⁰, in a review of recent advances in the chemistry and properties of atmospheric nucleants, decline to discuss the role of ions because of the lack of information on the primary chemical nature of the ions and, hence, on their modes of action; they exclude ions from the population of active condensation nuclei on the basis of the high water supersaturations necessary for the ions to become active. On the other hand, Montefinale *et al.*²⁰, suggest that ions, because they electrostrict conspicuous amounts of water molecules, may be responsible for the vertical transport of water under the influence of the electric gradients in the atmosphere. This transport may, in itself, have a significant role in effecting weather changes in response to geomagnetic events.

Because of the general lack of knowledge about the physical and chemical properties of the upper atmosphere, the questions concerning the nature of the mechanism linking geomagnetic disturbances and weather will most probably remain unanswered for some time. But we submit that the relationship between geomagnetic disturbances and weather warrants further investigation not only because of the short term implications but also because such studies may lead to an explanation of climatic changes on the geological time scale.

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¹ Uffen, R. J., *Nature*, **198**, 143 (1963).

² Uffen, R. J., *Proc. 22nd int. Geol. Congr., New Delhi* (1964).

³ Simpson, J. F., *Bull. geol. Soc. Am.*, **77**, 197 (1966).

- ⁴ Harrison, C. G. A., and Funnell, B. M., *Nature*, **204**, 566 (1964).
⁵ Opdyke, N. D., Glass, B., Hays, J. D., and Foster, J., *Science*, **154**, 349 (1966).
⁶ Watkins, N. D., and Goodell, H. G., *Science*, **156**, 1083 (1967).
⁷ Hays, J. D., *Bull. geol. Soc. Am.*, **82**, 2433 (1971).
⁸ Black, D. I., *Earth Planet. scient. Lett.*, **3**, 225 (1967).
⁹ Waddington, C. J., *Science*, **158**, 913 (1967).
¹⁰ Harrison, C. G. A., *Nature*, **217**, 46 (1968).
¹¹ Crain, I. K., *Bull. geol. Soc. Am.*, **82**, 2603 (1971).
¹² Wollin, G., Kukla, G. J., Ericson, D. B., Ryan, W. B. F., and Wollin, J., *Nature*, **242**, 34 (1973).
¹³ King, J. W., *Nature*, **247**, 131 (1974).
¹⁴ King, J. W., *Nature*, **245**, 443 (1973).
¹⁵ Roberts, W. O., and Olson, R. H., *J. atmos. Sci.*, **30**, 135 (1973).
¹⁶ Ninkovich, D., Opdyke, N., Heezen, B. C., and Foster, J. H., *Earth Planet. scient. Lett.*, **1**, 476 (1966).
¹⁷ Harrison, C. G. A., and Somayajulu, B. L. K., *Nature*, **212**, 5067 (1966).
¹⁸ Saylor, W. P., Wincer, D. E., Eiwien, C. J., and Carriker, A. W., *Space Radiation Guide*, (Technical Documentary Report AMRL-TDR-62-86, 1962).
¹⁹ Castleman, A. W., jun., *Space Sci. Rev.*, (in the press).
²⁰ Montefinale, A. C., Montefinale, T., and Papée, H. M., *Pure appl. Geophys.*, **91**, 171 (1971).

Equatorial undercurrent and climate in the Galapagos Islands

PALYNOLOGICAL data from sediment cores taken from El Junco crater lake on San Cristobal Island led to the recognition of stratigraphical fluctuations resulting from the water budget in the crater¹. The results show that the crater was dry between at least 34,000 and 10,000 yr BP. Colinvaux¹ suggested that the Galapagos Islands had a dry climate during that period, because the intertropical convergence zone (ITCZ) remained north of the geographical equator. On the other hand, Newell² showed that the ITCZ probably existed south of the equator at about that time (20,000 yr BP).

I demonstrate here that another mechanism could explain this general dryness.

The climate in the Galapagos Islands is influenced by regional and local hydrographic conditions which regulate the annual course of the temperature in the superficial waters. The most prominent features of the thermal properties in the Eastern Tropical Pacific are the upwellings: those which occur along the coast of the South American mainland, and those which are connected to the divergence along the equator. I have already shown³ that the local upwelling west of Isabela is a fluctuating but permanent feature which influences the temperature distribution throughout the archipelago. The annual thermal regime in the superficial waters of the islands is balanced between the warm season, when warm waters are drifting from the north and solar heating takes place, and a

cold season, when upwelling reaches maximum development.

Year to year comparisons of meteorological data and surface sea temperature, taken at the Charles Darwin Research Station (Bahia Academia, Santa Cruz Island)^{4,5}, have revealed that interannual variations in the thermal regime are similar to those recorded further east in the coastal waters along the Peruvian^{6,7} and Ecuadorian coast (G. Houvenaghel, unpublished). There is also a good correlation between the annual rainfall values from some localities on Santa Cruz Island, and the annual mean sea temperature measured in the surface waters at Bahia Academia (Fig. 1). The climate may be characterised using the pluviothermic quotient of Emberger. Calvet⁸ gives a formula for the computation of a pluviothermic quotient from the annual rainfall, R , the mean air temperature of the hottest, T , and the coldest, t , month, and the evaporation:

$$Q = 1,000 R/0.5 (T + t) (P_T - P_t)$$

where P_T and P_t are the water vapour pressure at temperatures T and t , respectively. Plotted against the mean annual sea temperature (Fig. 2), the computed values for Q also correlate well. Figure 2 shows that only a small decrease

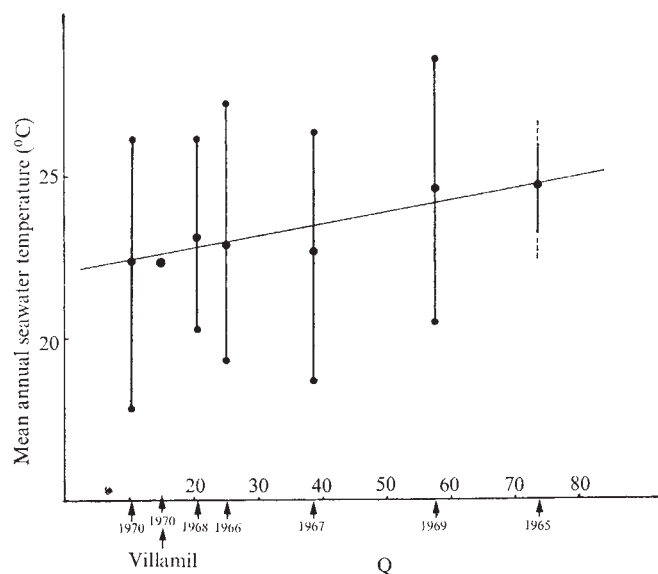


Fig. 2 The relationship between the mean annual seawater temperature and the annual pluviothermic quotient, Q , at Bahia Academia: $r = 0.91$ ($r = 0.87$, $P = 0.01$).

in the mean annual sea temperature is required to provide an arid or desert climate in the highlands of Santa Cruz. El Junco crater is located at an altitude of 650 m (ref. 1) which is intermediate between the highest two recording stations on Santa Cruz, and so the climate conditions prevailing on San Cristobal Island, 100 km ESE of Santa Cruz, must be similar to those recorded here. The dry climate which according to Colinvaux¹ existed in the past, could well have developed if a superficial cooling of sea water by about 1°C occurred at that time. Cooling can be expected to follow only a slight increase in the rate and duration of the upwelling (such as occurred, for example, during 1967) and it could last throughout the entire dry epoch. According to Bjerknes's model of equatorial circulation⁹, increases in both rate and duration of upwelling depend on the oceanwide stress of trade winds on the equator. Cooling in the Galapagos Islands may thus occur if the intensity of the field of the SE trade winds is increased. This could have been the case in the past: Newell² reports that the temperature gradient in both hemispheres was higher at 20,000 yr BP, and that the intensity of the Hadley cell circulation increased as well.

A dry climate in the Galapagos Islands could thus result

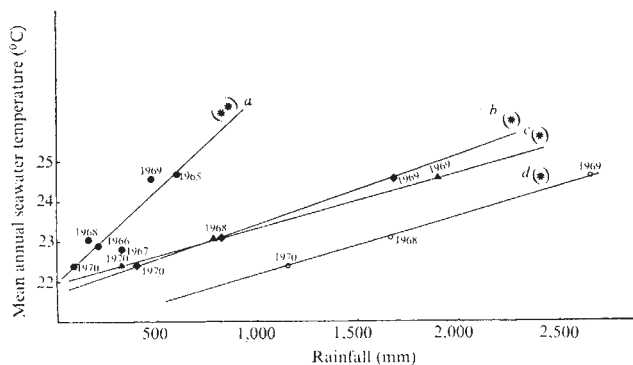


Fig. 1 The relationship between the mean annual seawater temperature, taken at Bahia Academia (Santa Cruz), and the rainfall collected in the same year at selected stations on Santa Cruz Island: ●, Bahia Academia (altitude, 6 m); ▲, Divine (320 m); ○, Media Luna (620 m); solid diamonds (200 m). a, $r = 0.903$; b, $r = 0.995$; c, $r = 0.996$; d, $r = 0.996$. (●*), $r = 0.875$, $P = 0.01$; (●*), $r = 0.990$, $P = 0.1$.

from changes in the intensity of the southern trade wind rather than from the shifting and continuing existence of the ITCZ in the Northern Hemisphere.

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¹ Colinvaux, P. A., *Nature*, **240**, 17-20 (1972).

² Newell, R. E., *Nature*, **245**, 91-92 (1973).

³ Houvenaghel, G., thesis, Univ. Libre de Bruxelles (1974).

⁴ *Meteorological Data*—Archives at the Charles Darwin Research Station, Bahia Academia, Santa Cruz.

⁵ Houvenaghel, G., *Mem. Acad. r. Sci. d'outre-mer Cl. Sci. nat. méd.* **49** **14** (1), 1-102 (1973).

⁶ Zuta, S., and Guillen, O., *Bol. Inst. Mar Peru*, **2** (5), 157-324 (1970).

⁷ Zuta, S., and Urquiza, W., *Bol. Inst. Mar Peru* **2** (8), 459-520 (1972).

⁸ Calvet, C., *Bull. Soc. Sci. nat. phys. Maroc*, **46**, 1-7 (1966).

⁹ Bjerknes, J., *Second Int. Oceanogr. Congr. Moscow*, **11**, 20 (1966).

Relative sizes of high and low spin states of atoms

STRUCTURAL studies with X rays^{1,2} of five and six-coordinated iron porphyrin complexes have shown that the distance from the iron atom to the nitrogen atoms of the porphyrin rings is less for low spin Fe(III) than for high spin Fe(III). As a result, it is often assumed that the Fe(III) atom is larger in its high spin state than in its low spin state. Similar size differences for the Fe(II) system form the basis of the Perutz model³ for the cooperativity of oxygen binding in haemoglobin. I discuss here the relative sizes of the high and low spin states of atoms in terms of the results of quantum mechanical calculations on isolated atoms. Contrary to earlier interpretations of structural studies, I have found that in the atoms I have studied the lowest lying high spin state of an atom in a given configuration is smaller than any state of lower spin.

Any theoretical discussion of the size of atoms must be concerned, in one way or another, with the radial density function $D(r_1)$ which is a measure of the probability of finding an electron at a distance r_1 from the nucleus. Unfortunately there is no unique definition of the size of an atom. In fact, many indices may be considered to be of atomic size. For example, the mean value of r_1 , denoted by $\langle r_1 \rangle$, is proportional to the average radial distance of an electron from the nucleus. Comparison of $\langle r_1 \rangle$ for two or more states of a given atom should indicate their relative radii. Also, as it is well known that the size of an atom is essentially determined by the outer shell⁴, the value of r_1 at the outer shell maximum of $D(r_1)$ could be taken as a measure of the size of an atom. Finally, the radius of a sphere containing a specified percentage of the total electronic charge is taken as a third index.

Consider the simple case of two electrons in a particular configuration in the field of a single nucleus. Figure 1 illustrates $D(r_1)$ for the singlet and triplet states arising from the $1s2s$ configuration of helium (the Pauli principle forbids a triplet state for the $1s^2$ configuration). The curves have been calculated for the moderately accurate correlated wave functions of Perkins⁵. The use of more accurate wave functions, such as those of Accad, Pekeris and Schiff⁶, which are nearly exact, would not alter the conclusions. On the basis of the indices listed in Table 1, it is clear that the helium atom is distinctly smaller by as much as 19% in the high spin 2^3S state than in the 2^1S state.

Similar calculations with the Perkins wave functions for Be^{2+} show that the 2^3S state is smaller than the 2^1S state by as much as 10%. (One curious result should be noted: in both He

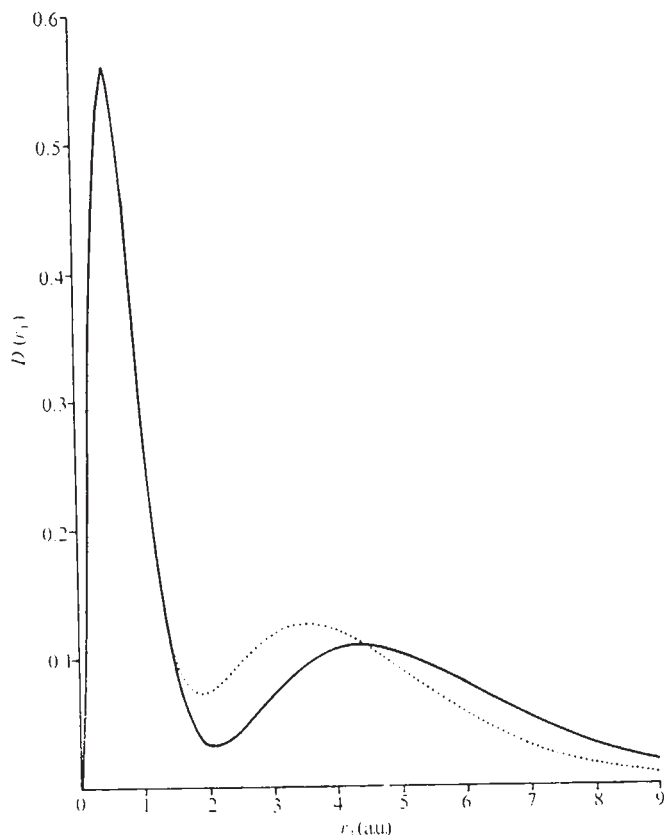


Fig. 1 Radial density function $D(r_1)$ in the 2^3S (dotted line) and 2^1S (solid line) states of helium.

and Be^{2+} the value of r_1 at the L shell maximum of $D(r_1)$ corresponds to the radius of a sphere containing ~69% of the electronic charge in both the 2^3S and 2^1S states.) Very accurate values of $\langle r_1 \rangle$ have been reported⁶ for the singlet and triplet states of the $1sns$ and $1snp$ ($n = 2-5$) configurations of the helium isoelectronic sequence up to $Z = 10$. In every case, the triplet state $\langle r_1 \rangle$ is greater than the corresponding singlet value and, therefore, it must be concluded that for atoms with two electrons a high spin atom is smaller than a low spin atom with the same configuration and nuclear charge.

For atoms with many electrons, only a few wave functions have been reported with an accuracy approaching that obtained with atoms which have only two electrons. It is, however, well known that the Hartree-Fock method gives good charge distributions. Also, because the size of an atom is essentially determined by the outer shell, the size of atoms with many electrons can be discussed in terms of the radial density associated with the outermost orbital, in much the same way as atoms with two electrons have been discussed in terms of $D(r_1)$. Assuming that, and using Clementi's Hartree-Fock wave functions⁷, a high spin atom is found to be smaller than a low spin atom with the same configuration and nuclear charge, for all the states arising from the p^2 , p^3 and p^4 configurations of the partially filled L and M shells. The conclusion is valid for large atomic numbers. For example, qualitatively identical results are obtained for the 4S and 3P states of the $\text{K}(2) \text{L}(8) 3s^2 3p^3$ configuration of both P and Kr^{21+} .

Unfortunately, Clementi's tables⁷ include only the lowest state of the $\text{K}(2) \text{L}(8) \text{M}(8) 3d^n$ configurations and, therefore,

Table 1 Relative radii of the 2^3S and 2^1S states of helium (a.u.)

Index	2^3S	2^1S
$\langle r_1 \rangle$	2.55	2.97
L shell maximum	3.54	4.38
99% charge	8.75	10.23
95% charge	6.78	7.90
90% charge	5.67	6.75
80% charge	4.49	5.42
65% charge	3.24	3.98

cannot be used to discuss the spin states of biological interest. It would, however, be very surprising if results similar to those observed for p^n configurations were not also found for d^n configurations. This conjecture is supported by some results⁸ for the $K(2) L(8) 3s^2 3p^6 3d^1 4d^1$ configuration of Ca. The 4d radial densities indicate that Ca is smaller in the high spin 3F state than in the low spin 1S , 1D and 1G states. In this case, unlike the p^n configurations, there is a second allowed state which has the maximum multiplicity. Thus, the 3P state lies above the 3F and 1D states and leads to a correspondingly larger atomic radius.

Although it has long been realised that the effective radius of an atom depends on its electronic structure and environment⁹, it seems that the observations of free atoms discussed here have not been reported previously. Of course, environmental effects can be large and can lead to results opposite to those found in free atoms; a detailed discussion of environmental effects is, however, beyond the scope of this article.

Although these results are likely to be contrary to the 'chemical intuition' of many scientists, it should be noted that electron repulsion is greater in the triplet state than in the singlet state of some two-electron systems¹⁰ contrary to the usual interpretation of Hund's rule.

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- Hoard, J. L., in *Structural Chemistry and Molecular Biology*, (edit. by Rich, A., and Davidson, N.), (Freeman, San Francisco, 1968).
- Countryman, R., Collins, D. M., and Hoard, J. L., *J. Am. chem. Soc.*, **91**, 5166-5167 (1969).
- Perutz, M. F., *Nature*, **228**, 726-734 (1970).
- Coulson, C. A., *Valence*, second ed., 39 (Oxford University Press, London, 1961).
- Perkins, J. F., *J. chem. Phys.*, **39**, 687-693 (1963).
- Accad, Y., Pekeris, C. L., and Schiff, B., *Phys. Rev.*, **A4**, 516-536 (1971).
- Clementi, E., *IBM JI Res. Dev., Suppl.* 9 (1965).
- Froese Fischer, C., *J. Phys.*, **B5**, 1302-1307 (1972).
- Pauling, L., *Nature of the Chemical Bond*, third ed., 224 (Cornell University Press, Ithaca, 1960).
- Boyd, R. J., and Coulson, C. A., *J. Phys., B (GB)*, **6**, 782-793 (1973), and references therein.

Significance of the even-carbon n -paraffin preference of a Spanish crude oil

It has been suggested that processes other than the accumulation of biogenic hydrocarbons play an important role in the organic formation of petroleum. That can be inferred from a comparison of the different types of organic compounds found in sediments and petroleum^{1,2}.

Those studies dealt mainly with saturated hydrocarbons, among which n -paraffins have a special geochemical significance. That is because their distribution depends on the degree of maturity and the nature of the source material.

Most recent sediments exhibit an n -paraffin distribution concentrated between C_{18} and C_{32} , characterised by a marked predominance of odd numbers of carbon atoms ($CPI > 1$), (refs 3 and 4). As a result of physicochemical maturation processes, this predominance diminishes in the oldest sediments, and disappears in petroleum in which there is a shift in the maximum of the n -paraffin distribution curve towards lower carbon numbers. Thus, the CPI of a sediment is considered as an indication of its capacity for generating hydrocarbons³, although both the extent of generation of hydrocarbons and the type of source material, influence the distribution of the n -alkanes found in ancient sediments⁵.

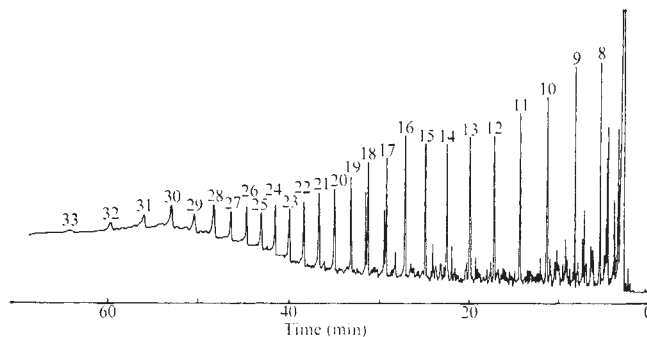


Fig. 1 Gas chromatogram of the Amposta-marino crude oil (OV-101 capillary column).

Smooth n -paraffin distributions ($CPI \approx 1$) are typical of marine organic facies. A relative predominance of high molecular weight n -paraffins, with possibly an odd carbon preference in the C_{20} - C_{30} range has, however, been invoked as evidence of non-marine origin in some sediments^{4,6} and crude oils⁷⁻⁹. A slight predominance of odd number n -paraffin is not definite proof of continental origin, because it may have arisen by diagenetic processes¹⁰. The predominance of n -paraffins with odd numbers of carbon atoms has also been claimed to support the hypothesis of fatty acid decarboxylation in the generation of n -paraffins.

In our work on the chemical characterisation of the crude oils from the first oil field in production in the Mediterranean (near the mouth of the Spanish Ebro River), we have found that the Amposta-marino crude (Mesozoic, 16.6 API) apparently exhibits a marked predominance of n -paraffins with even numbers of carbon atoms within the C_{20} - C_{34} range (Fig. 1). That is interesting not only geochemically but also because it provides a means for the characterisation of accidental spillage of the crude oil in the sea¹¹.

We isolated the n -paraffins by separate treatment of the light distillates ($< 200^\circ C$) and of the desasphalted residue with 5 Å molecular sieves. Analysis by gas-liquid chromatography provided the real distribution of n -paraffins in the crude oil (Fig. 2). A maximum appeared at $n=8$, and a CPI value of 0.74 was observed in the range C_{24} - C_{34} .

Slight predominances of sedimentary n -paraffins with even number of carbon atoms have been reported^{4,12-14}. Considering the n -paraffin distributions found in living organisms (odd number preference), the even number predominances should be a result of post-depositional reductive processes acting on the organic matter, that is, hydrogenolysis of fatty acids^{4,12} and alcohols¹⁵. There is experimental evidence of such geochemical transformations¹⁶⁻¹⁸.

Even-carbon n -paraffins can also be formed by the condensation of two fragments originating from fatty acid decarboxylation or from n -paraffin thermal degradation^{19,20}. For thermodynamic reasons, however, these processes should be of secondary importance in comparison with true degradation.

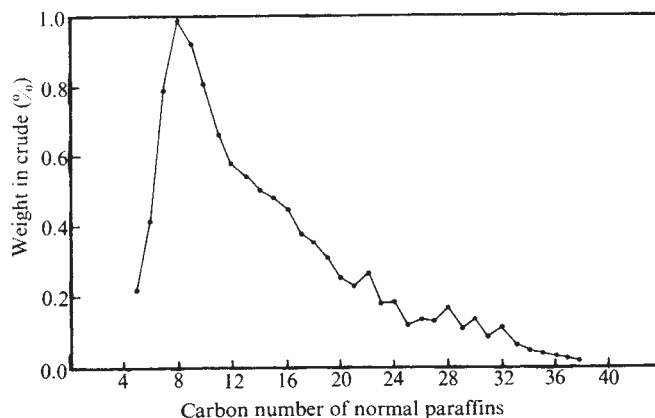


Fig. 2 Distribution of n -paraffin in the Amposta-marino crude.

As far as petroleum is concerned only a very few cases of even-carbon predominances have been reported²¹. The most remarkable was found in a Nigerian crude oil within the C₄₀–C₄₆ range²². Its origin is very difficult to explain by the processes already mentioned.

To the best of our knowledge, there are no previously described examples of such a clear even-carbon number predominance within the C₂₀–C₃₄ range in crudes, as that exhibited by the Amposta-marino oil. The type of distribution shown in Fig. 2 leads to the assumption that the process of formation is not secondary but rather that the even-carbon number predominance is produced by the reduction of organic material containing large amounts of long chain fatty acids or alcohols. This could indicate a continental character in the crude oil, as would be expected for a deltaic environment. As long as no definite preference is observed in the C₁₂–C₁₈ region, the reduction of alcohols seems to be the more probable process.

Such a reduction has already been invoked, for example, to explain the formation of steranes from sterols in petroleum¹². Furthermore, the *n*-C₂₂ and *n*-C₂₈ paraffins, the principal hydrocarbons of the C₂₀–C₃₄ range studied, have been considered to be related geochemically to *n*-docosanol² and *n*-octacosanol¹⁵, the main components of some continental plants and sediments. On the other hand, the formation of even-number *n*-paraffins by the reduction of waxy alcohols could explain, in some cases²⁴, the balanced distribution of C₂₀–C₃₀ *n*-paraffins in old sediments and crude oils: it may result from dilution of the pre-existing odd-number compounds.

It is worth pointing out, in connection with these reduction processes, that phytane is more abundant than pristane in this crude (phytane/pristane=1.4), although it does not seem necessary to invoke a reduction process in order to explain this predominance, because of the existence of isoprenoid alkanes in living organisms.

In short, chemical and physical environments must be as important as time in establishing differences in the *n*-paraffin distributions. In particular, the manifest existence of reduction processes during the formation of the Amposta-marino crude affords direct evidence of the need for a reducing condition in the sediments for the formation of petroleum. This idea has been supported until now by the absence of olefins and other easily reducible substances in crudes. In any case, the mechanism of such processes has not yet been established.

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- ¹ Philippi, G. T., *Adv. org. Geochem.*, **25** (1968).
- ² Ourisson, H., and Albrecht, P., *Angew. Chem., int. Ed. Engl.*, **10**, 209 (1971).
- ³ Bray, E. E., and Evans, E. D., *Bull. am. Ass. Petrol. Geol.*, **49**, 248 (1965).
- ⁴ Kvenvolden, K. A., *Adv. org. Geochem.*, 335 (1966).
- ⁵ Koons, C. B., Jamieson, G. W., and Ciereszko, L. S., *Bull. Am. Ass. Petrol. Geol.*, **49**, 301 (1965).
- ⁶ Rogers, M. A., and Koons, C. B., *Adv. Chem. Ser.*, **103**, 67 (1971).
- ⁷ Martin, R. L., Winters, J. C., and Williams, J. A., *Nature*, **199**, 110 (1963).
- ⁸ Didyk, B. M., and McCarthy, E. D., *Nature*, **232**, 103 (1971).
- ⁹ Reed, K. J., *Bull. Am. Ass. Petrol. Geol.*, **53**, 1502 (1969).
- ¹⁰ Albrecht, P., thesis, Univ. Strasbourg (1969).
- ¹¹ Albaigés, J., *Oilgas*, **75**, 73 (1974).
- ¹² Welte, D. H., and Ehardt, G., *Geochim. cosmochim. Acta*, **32**, 465 (1968).
- ¹³ Han, J., McCarthy, D., Van Hoven, W., Calvin, M., and Bradley, W. H., *Proc. Natn. Acad. Sci., U.S.A.*, **59**, 29 (1968).
- ¹⁴ Schenk, P. A., *Adv. org. Geochem.*, 261 (1968).
- ¹⁵ Knoche, H., Albrecht, P., and Ourisson, G., *Angew. Chem., int. Ed. Engl.*, **7**, 631 (1968).
- ¹⁶ Blumer, M., *Science*, **149**, 722 (1965).
- ¹⁷ Mitterer, R. M., and Hoering, T. G., *Y6. Carnegie Instn Wash.*, 510 (1966).

- ¹⁸ Bayliss, G. S., *Am. chem. Soc. Div. Petrol. Chem.*, **13**, F117 (1968).
- ¹⁹ Robinson, R., *Nature*, **212**, 1291 (1966).
- ²⁰ Jürg, J. W., and Eisma, E., *Science*, **144**, 1451 (1964).
- ²¹ Henderson, W., Eglinton, G., Simmonds, P., and Lovelock, J. E., *Nature*, **219**, 1012 (1968).
- ²² Brunnock, J. V., *Nature*, **212**, 385 (1966).
- ²³ Speers, G. C., and Whitehead, E. V., *Adv. org. Geochem.*, 638 (1968).
- ²⁴ Oró, J., Nooner, D. W., and Wihström, S. A., *Science*, **147**, 870 (1965).

Age of valley deposits in Périgord

In Périgord, as in other parts of Europe, climatic and ecological variations inferred from Pleistocene sediments and organic remains have long provided the basis for correlating stratified Palaeolithic artefacts¹ and for subdividing the geological record^{2,3}. Radiocarbon dating now makes it possible to establish independent environmental, archaeological and palaeontological sequences and thus to investigate the links between them. I report here the results of work on valley deposits undertaken with this end in view; their archaeological and palaeoclimatic implications will be considered elsewhere.

Near their junction, the Vézère and Dordogne rivers and their larger tributaries (Fig. 1) display traces of three post-Tertiary depositional episodes separated by periods of erosion. Names are proposed for the resulting rock units (Fig. 2) to simplify discussion.

The 'Condat Tufa' is a localised deposit consisting of marls and tufas. Five ¹⁴C dates were obtained at various levels in the type section (section I), where the formation attains a maximum observed thickness of 8.2 m and overlies Jurassic limestones. (To judge from the published description this is the tufa at Coly from which Bourdier⁴ reported a temperate molluscan fauna.) The dates (Table 1) are consistent with their relative stratigraphical position and when plotted against depth indicate a uniform depositional rate of about 21 cm per 1,000 yr; but being on tufa they are automatically suspect. Variations in δ¹³C do not necessarily provide a good measure of the limestone dilution effect⁵ and in the present case yield no consistent pattern; and, as tufa is not forming today, a correction based on current ¹⁴C: ¹²C ratios could not be introduced. As regards contamination by younger carbon, pretreatment with acid washes was supplemented by various analyses of the three uppermost samples. Although thin sections reveal no obvious evidence of recrystallisation, and X-ray diffraction shows that all three samples consist of calcite with less than 4 mol% MgCO₃. JD-22 displays some scattered rhombs of dolomite and signs of secondary calcitic cementation (P. Wigley, personal communication). This supports the inference that the high porosity (15–30% void ratio) of JD-21 and 22 coupled with their position at the surface of the tufa would have exposed them to ¹⁴C exchange, and thus argues for their rejection. Sample JD-2, being compact and rich in algal material, is more likely to yield a date which refers to the time of deposition.

The 'Montignac Formation' includes scree, colluvium and alluvium, and ranges in lithology from gravel to clay. Its surface slope attains 30° at some valley margins and decreases to form horizontal terraces along the larger streams, such as the Vézère at Montignac; its thickness commonly exceeds 25 m. The deposit is separated from the Condat Tufa by a weak unconformity; elsewhere it rests on Tertiary or older rocks. Although some aggradation may have taken place before the end of tufa deposition (unlike JD-2, samples JD-21 and JD-22 contain quartz grains), the bulk of the Montignac phase presumably postdates 21,730 BP. Derived Middle and Advanced Palaeolithic artefacts were found in the Montignac deposits at various exposures; at the Magdalenian open site of Solvieux, on the

Table 1

Rock unit	Sample No.	Laboratory No.	Grid reference ¹	Location	Age (y BP)	¹³ C: ¹² C Rel PDB	Material	Remarks
Condat Tufa	JD-21	I-6945	513.6 × 313.2 (Section I)	1°14'16"E 45°7'0"N	12,890 ± 175	—	Porous Tufa	At contact with Montignac Fm
Condat Tufa	JD-22	I-6946	513.6 × 313.2 (Section I)	1°14'16"E 45°7'0"N	12,320 ± 175	-8.4	Nodular Tufa	At contact with Montignac Fm
Condat Tufa	JD-2	I-6795	513.6 × 313.2 (Section I)	1°14'16"E 45°7'0"N	21,730 ± 410	-10.3	Algal Tufa	2 m below contact
Condat Tufa	JD-93	I-7472	513.6 × 313.2 (Section I)	1°14'16"E 45°7'0"N	25,880 ± 650	-9.2	Dendritic Tufa	3 m below contact
Condat Tufa	JD-94	I-7473	513.6 × 313.2 (Section I)	1°14'16"E 45°7'0"N	31,050 ± 1500	-11.7	Marl	4 m below contact
Limeuil Alluvium	DP-5	I-6794	494.5 × 296.3 (Section V)	1°0'5"E 44°57'21"N	1,335 ± 90	-22.2	Charcoal	Age normalised for fractionation 1,375 ± 90

¹Grid references refer to Carte de France (1/25,000).

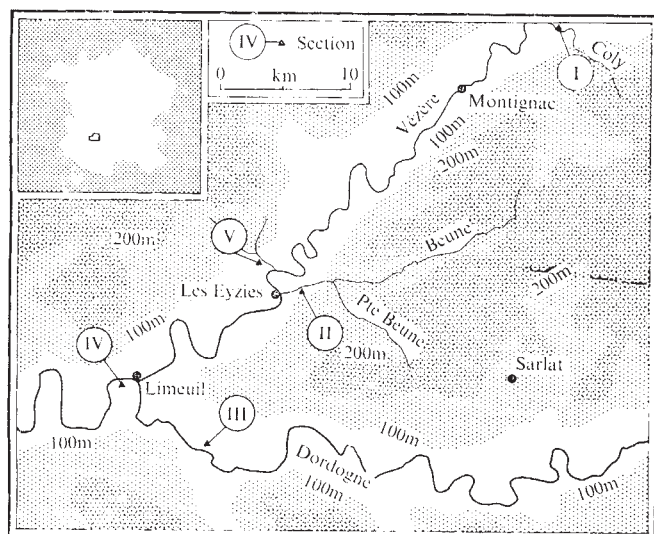


Fig. 1 Location map of places mentioned in text.

Isle (which joins the Dordogne west of the main area of study), deposition was apparently still in progress during the period of occupation. Radiocarbon dates for comparable material from the caves and shelters of Périgord⁶ suggest that deposition of the Montignac Formation continued until about 10,000 yr ago. It is worth noting that in the Beune valley, where the Montignac deposits are coarse in texture and slope down beneath the fine-grained material in which the river is now incised, a ¹⁴C date of 9,040 ± 60 yr BP (Grn-4492) has been obtained at a depth of about 13.7 m slightly above the contact between silty muds and the underlying sands and gravels⁷.

The 'Limeuil Alluvium' generally underlies the plains that border the larger streams, although it may be concealed by modern flood deposits. It consists principally of silty sand (whereas the flood material is dominated by coarse sand with abundant pebbles), and attains a maximum observed thickness of 7.5 m at Limeuil (section IV). Roman potsherds have been found within the deposit here as well as in a quarry west of Coux-et-Bigaroque (section III) and in the Beune opposite the cave of Font de Gaume (section II). Radiocarbon dating of the Limeuil Alluvium in the Manaurie valley (section V; see Table 1) has confirmed its historical age.

The resulting sequence may be summarised as follows. Localised tufa deposition came to an end about 21,000 yr ago. It was closely followed by a phase of scree formation, slope wash and valley filling. About 10,000 yr ago stream incision supervened. Renewed aggradation took place during or after Roman times, to be supplanted by the current erosional episode at some stage after AD 575.

Field mapping shows that the Montignac Formation of the present account includes most of the deposits attributed on the geological sheets of the area⁸ to the lower terrace of the *alluvions anciennes* as well as part of the *alluvions modernes*; the rest of the *alluvions modernes* correspond to our Limeuil Alluvium. Comparison with the accounts of Fénelon² and Bourgon³ shows similar discordances.

Chaput⁹ has described a deposit in the Garonne near its junction with the Dordogne similar in morphology and archaeological content to the Montignac Formation and linked to moraines at the foot of the Pyrenees which he ascribes to the Würm glaciation; the deposition of the Montignac Formation during glacial times, already suggested by its wealth in presumably frost-riven scree material, is further supported by the ¹⁴C dates of 17,500 and 9,700 BP obtained for peats laid down behind two of the outermost Pyrenean moraines¹⁰. The apparent absence in the area under review of valley deposits demonstrably older than the Condat Tufa, though surprising in view of the very long and complex sequence of glacial episodes that has been identified in the caves of Périgord¹¹, accords with the dearth of evidence for pre-Würm glaciations in the Pyrenees¹⁰ and with the finding that only one, 'rather recent', glacial phase is represented in the upper Dordogne¹². Moreover, neither Fénelon nor Bourgon succeeded in identifying slope deposits older than the Würm^{2,3}. As in the Mediterranean Basin¹³, we can either postulate that evidence for earlier glacial episodes has been destroyed¹⁴ or take the field record at its face value when attempting climatic reconstructions.

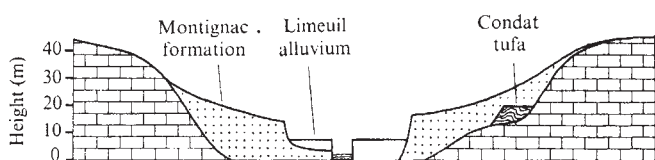


Fig. 2 Schematic section of valley fills.

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¹ Bordes, F., in *The Explanation of Culture Change* (edit. by Renfrew, C.), 217-226 (Duckworth, London, 1973).

- ² Fénelon, P., *Le Périgord. Étude morphologique*, 526 (Lahure, Paris, 1951).
- ³ Bourgon, M., *Archives Inst. Paléont. Hum., Paris, Mém.* 27, 141 (1957).
- ⁴ Bourdier, F., *Soc. préhist. Fr.*, **42**, 173 (1945).
- ⁵ Bowler, J. M., and Polach, H. A., in *Paleopedology* (edit. by Yaalon, D. H.), 97–108 (International Society of Soil Science and Israel Universities Press, Jerusalem, 1971).
- ⁶ Coles, J. M., and Higgs, E. S., *The Archaeology of Early Man*, 454 (Faber and Faber, London, 1969).
- ⁷ Donner, J. J., *Pollen et Spores*, **11**, 97–116 (1969).
- ⁸ *Carte Géologique de la France (1/80,000)*, Sheets 182 and 183.
- ⁹ Chaput, E., *Bull. Soc. géol. Fr.*, **24**, 449–461 (1924).
- ¹⁰ Taillefer, F., *Bull. Ass. Fr. Ét. Quat., Suppl.*, 19–32 (1969).
- ¹¹ Laville, H., *Bull. Ass. Fr. Ét. Quat., Suppl.*, 77–80 (1969).
- ¹² Veyret, Y., *Bull. Ass. Fr. Ét. Quat.*, **9**, 21–30 (1972).
- ¹³ Vita-Finzi, C., *Nature*, **224**, 173 (1969).
- ¹⁴ Fairbridge, R. W., in *The Mediterranean Sea* (edit. by Stanley, D. J.), 99–113 (Dowden, Hutchinson and Ross, Stroudsburg, 1971).

Grassland species can influence the abundance of microbes on each other's roots

NEIGHBOURING plants usually influence each other's growth. These interactions are very important in determining the composition and structure of plant communities, but much remains to be discovered about the mechanisms by which plants influence each other. Often plants compete for requirements such as light and mineral nutrients. Some plants exude substances which are toxic to others^{1–3}. Another possibility, as yet unexplored, is that interactions occur through changes in the microbial populations on the root surface or in the rhizosphere (the soil near the root surface). It is known that rhizosphere and root surface microorganisms can affect the plant's nutrient uptake and growth^{4,5}, but up to now there has been no evidence on whether plant species which naturally grow together can influence each other's microbial populations. We present here such evidence for two species common in British lowland grassland, *Lolium perenne* L. (perennial ryegrass) and *Plantago lanceolata* L. (ribwort plantain, a dicotyledonous species).

Seeds of the two species were collected from a permanent grassland site near Bristol. Soil was collected from a neighbouring field where both species also occur. The soil was mixed and placed in pots 13 cm in diameter; it was not sterilised, nor was fertiliser added. Seeds were germinated in vermiculite, and four seedlings were planted in each pot, either four *Lolium*, four *Plantago*, or two of each species.

Two similar experiments were performed. In the first there were nine replicate pots per treatment, and in the second ten. In the first experiment the plants grew from July until September 1973, and in the second from November 1973 to February 1974. The plants grew in a glasshouse for 2–3 months, and the roots were then washed free of soil. Randomly selected root segments were stained with phenyl acetic aniline blue and the abundance of bacteria and fungi on the root surface was determined by direct observation under a microscope: the percentage of the root area covered by bacteria was estimated in numerous randomly-positioned squares, and the length of fungal mycelium per unit root surface area was determined by the line intersection method⁶. The techniques are described in more detail elsewhere⁷. In the second experiment only fungi were determined.

Table 1 summarises the results. In every case the microbial abundance was greater when the two species were growing together than when they were separate, and by analysis of variance this main effect (separate/together) was always statistically significant. It is true that by Duncan's multiple range test only *Lolium* showed a significant increase in microorganisms in experiment 1, and only *Plantago* did so in experiment 2. Nevertheless, both species always showed the trend in the same direction.

We have no direct evidence on how (if at all) the increase in root surface microorganisms influences the higher plants, but the dry weights, shown in Table 2, are relevant. In experiment 1,

Table 1 Percentage cover of bacteria, and fungal mycelium length per unit root surface area, on root surfaces of *Lolium perenne* and *Plantago lanceolata* grown either separately or together

	Bacteria		Fungi			
	Cover %		Mycelium length (mm mm ⁻²)			
	Experiment 1	Experiment 2	Experiment 1	Experiment 2	Experiment 1	Experiment 2
	<i>Lolium</i>	<i>Plantago</i>	<i>Lolium</i>	<i>Plantago</i>	<i>Lolium</i>	<i>Plantago</i>
Separate	*4.3 a	5.6 ab	0.7 a	1.8 ab	8.4 a	8.8 a
Together	6.3 b	5.8 b	2.1 b	2.9 b	11.3 a	15.5 b
Significance†						
Separate/	<i>P</i> < 0.025		<i>P</i> < 0.01		<i>P</i> < 0.001	
together						
Between	ns		<i>P</i> < 0.025		ns	
species						
Interaction	ns		ns		ns	

* Within each group of four, figures not followed by the same letter are significantly different (*P* < 0.05) by Duncan's multiple range test.

† Results of analysis of variance; ns, not significant.

Lolium showed an increased shoot weight in the mixture, while *Plantago* showed no change. The total yield by the mixture exceeded that of either pure stand (Table 2b). In experiment 2 there was a similar increase of weights in the mixture, though it was not quite statistically significant. These results differ from the commonest situation in direct competition, where increased growth by one species involves reduced growth by another. They could be caused by the increased microbial population having a beneficial effect on plant growth. Yield increases by mixtures, of the sort shown in Table 2b, have been reported previously, but do not always occur^{8–10}. A recent survey of the literature on two-species and two-variety mixtures in pot and field experiments (E.I.N., unpublished), found that in 20% of cases the mixture yielded more than either pure stand. No satisfactory explanation has yet been offered as to why some mixtures show this increase and others do not, and it is possible that the nature of the microbial interactions is one of the factors determining this.

Table 2 Dry weight of shoots (g). Statistical significance expressed as in Table 1

<i>a</i> Mean weight per plant*.				
	Experiment 1		Experiment 2	
	<i>Lolium</i>	<i>Plantago</i>	<i>Lolium</i>	<i>Plantago</i>
Separate	0.71 a	0.69 a	0.54 a	0.56 a
Together	1.01 b	0.69 a	0.71 a	0.62 a
Significance				
Separate/together	<i>P</i> < 0.025		ns	
Between species	<i>P</i> < 0.01		ns	
Interaction	<i>P</i> < 0.025		ns	
<i>b</i> Mean weight per pot (four plants)*.				
	<i>Lolium</i> alone	<i>Plantago</i> alone	Mixture	Significance
Experiment 1	2.84 a	2.75 a	3.39 b	<i>P</i> < 0.025
Experiment 2	2.17 a	2.26 a	2.65 a	ns

*Letters (a and b) are for comparisons within each row.

Clearly the significance of our findings for higher plant growth is at present speculative. What we have definitely shown is that two plant species which commonly grow together can influence each other's root surface microbial population.

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¹ Muller, C. H., *Vegetatio*, **18**, 348–57 (1969).

- ² U.S. National Committee for the International Biological Program, *Biochemical Interactions among Plants* (National Academy of Sciences, Washington, D.C., 1971).
- ³ Whittaker, R. H., and Feeny, P. P., *Science*, **171**, 757-70 (1971).
- ⁴ Barber, D. A., *A. Rev. Pl. Phys.*, **19**, 71-88 (1968).
- ⁵ Bowen, G. D., and Rovira, A. D., in *Root Growth* (edit. by Whittington, W. J.), 170-99 (Butterworths, London, 1969).
- ⁶ Newman, E. I., *J. appl. Ecol.*, **3**, 139-45 (1966).
- ⁷ Rovira, A. D., Newman, E. I., Bowen, H. J., and Campbell, R., *Soil Biol. Biochem.* (in the press).
- ⁸ Syme, J. R., and Bremner, P. M., *J. appl. Ecol.*, **5**, 659-74 (1968).
- ⁹ Marshall, D. R., and Jain, S. K., *J. Ecol.*, **57**, 251-70 (1969).
- ¹⁰ Harris, W., and Thomas, V. J., *N.Z.J. agric. Res.*, **15**, 19-32 (1972).

Catalysomes of adipose tissue are artefacts of enzyme localisation

WHEN adipose tissue (insect fat body and gut, mouse mesentery) is stained to localise esterases (using 5-bromoindoxyl acetate as indigogenic substrate³) or dehydrogenases (using various substrates and nitro-blue tetrazolium⁷) the reaction product forms a single cap on each lipid droplet^{8,9,11-13}. The cap has been thought to be an organelle¹², perhaps a specialised mitochondrion concerned in lipid metabolism², for which Wigglesworth proposes the term catalysome. Although the localisation of the reaction products in caps is very precise, the nature of the organelle has remained a mystery. In *Calpodes* fat body, 5-bromoindoxyl acetate always gives one perfect blue cap of the indigo reaction product for each lipid droplet (Fig. 1a) and similar localisation is obtained with the dehydrogenases (Fig. 1b), but further studies have shown that the catalysomes are artefacts caused by the preferential precipitation of reaction products in lipid or at lipid-water interfaces.

Our initial intention was to locate the caps by electron microscopy, and thus discover the nature of the organelle. In *Calpodes* larvae, the fourth instar lipid droplets disappear and are replaced by a new generation within 24 h of ecdysis to the fifth instar (ref. 5). Esterase caps can be demonstrated from the moment that the new generation of lipid droplets becomes visible by light microscopy, that is when they are about the size of mitochondria. Electron microscopy (EM), however, showed no organelle preferentially associated with lipid droplets at this time when such an association should have been unmistakable. Esterase localisation for EM by the 8-acetoxyquinoline/bismuth oxynitrate method of Deimling¹ also failed to identify any esterolytic organelle associated with lipid droplets. Bismuth was deposited in mitochondria spread throughout the cytoplasm, and within some droplets (Fig. 2) but no cap-like organelle could be discerned. The bismuth precipitate associated with lipid which in EM sections often appeared to spread from one or occasionally two foci on the periphery of a droplet (Fig. 2) was seen in whole mounts as a large number of spots on the surface of each droplet (Fig. 1c).

Our failure to locate the catalysome as well as the rather different intracellular localisation of esterase reaction product demonstrated by Deimling's method suggested that the validity of the methods that produced the original cap localisations should be checked. An alternative substrate for the indigogenic demonstration of esterases, 5-bromo-4-chloroindoxyl acetate, has greater affinity for protein which causes it to be retained more strongly at the site of esterase activity⁴. The cytoplasm of fat body cells reacted intensely when this substrate was applied (Fig. 1d), but there was no localisation in lipid or caps. The localisation of esterase reaction products in caps may therefore be an artefact resulting from lipid solubility of the indoxyl intermediate in the indigogenic reaction⁴. An attempt was made to reproduce the artefact on free lipid droplets by mixing them with esterase *in vitro*. Fat bodies were homogenised

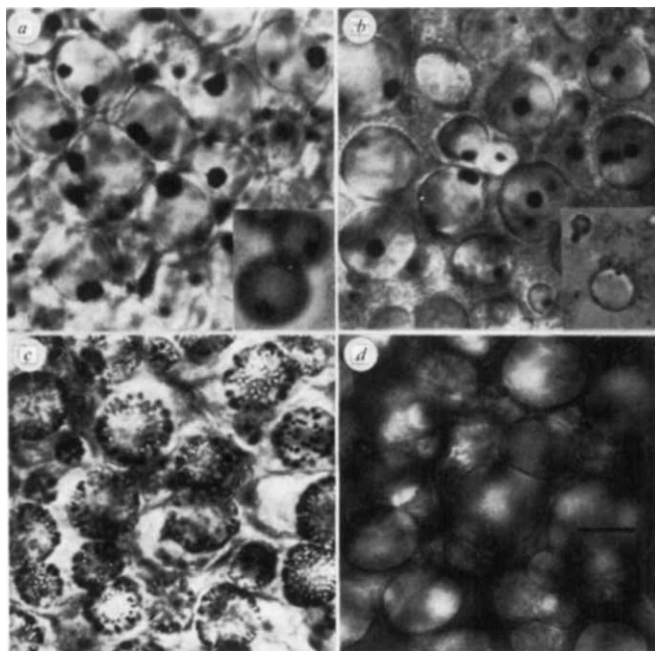


Fig. 1 Distribution of enzyme reaction products in whole mounts of *Calpodes* fat body: Scale 10 μ m. *a*, Caps of indigo on lipid droplets from esterase demonstration with 5-bromoindoxyl acetate. Insert, indigo caps on olive oil droplets. *b*, Caps of diformazan on lipid droplets from NADH diaphorase demonstration using Nitro B.T. Insert, caps of diformazan on olive oil droplets. *c*, Surface spots of reaction product on lipid droplets from esterase demonstration by Deimling's method. *d*, Cytoplasmic indigo precipitate with no lipid association from esterase demonstration with 5-bromo-4-chloroindoxyl acetate.

in buffer and the homogenate centrifuged at 70,000g for 1 h to produce a discrete fat layer above a supernatant and pellet. The supernatant and pellet were taken from below the fat layer and added to the indigogenic reaction mixture (substrate 5-bromoindoxyl acetate) together with a small amount of olive oil which was dispersed by stirring. After 1 h the numerous lipid droplets less than 14 μ m in diameter all carried one indigo cap similar in appearance to those produced in intact fat body (Fig. 1a, insert). No indigo precipitates were obtained in a control mixture lacking esterase. Repetition of the experiment adding only the supernatant to the indigogenic reaction mixture also produced single caps on droplets.

Since esterase localisation in caps seems to be an artefact, the similar localisation of various dehydrogenases must also be questioned. Eight dehydrogenases have been reported to be localised in the caps on lipid droplets¹². But since the final reaction in all of these histochemical tests involves a common enzyme 'Nitro B.T. reductase' the existence of an artefact common to all reaction mixtures can be decided using a mixture for a single dehydrogenase. We chose to investigate the most active, NADH diaphorase. A homogenate of *Calpodes* fat body was prepared as described previously and two fractions taken from it, the supernatant and a mixed supernatant and pellet. These were added to the NADH diaphorase reaction mixture. After 1 h caps of formazan had formed on many lipid droplets in the mixture containing pellet and supernatant (Fig. 1b insert). In contrast to the indigogenic result two or more caps per droplet was the most common situation which suggests a difference from the localisation reported in tissue. In our experiments, however, incubations of live fat body to demonstrate NADH diaphorase generally resulted in a small number of caps per lipid droplet (Fig. 1b). No caps were produced on lipid droplets in the supernatant fraction, a predictable result since NADH diaphorase is considered to be mitochondrial. These experi-

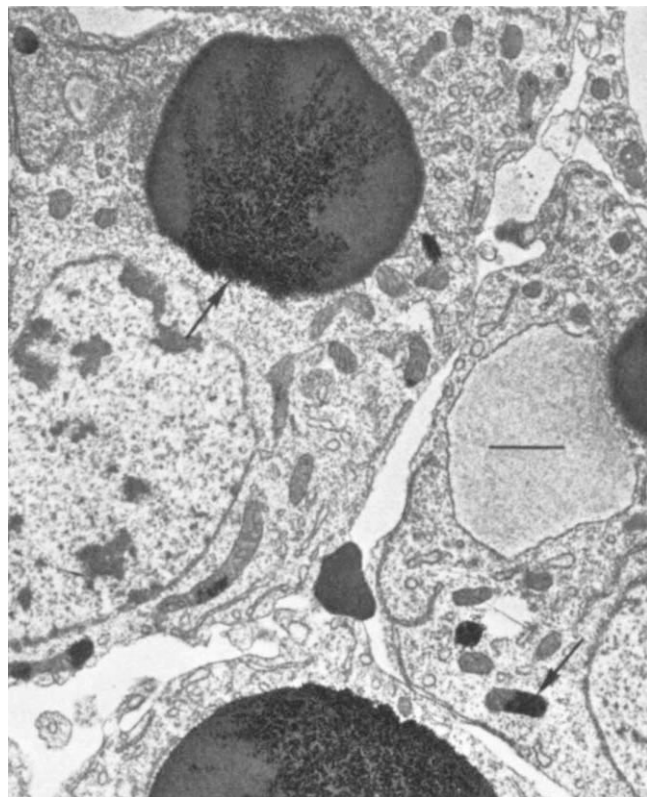


Fig. 2 Reaction product (arrowed) localised in mitochondria and lipid droplets in *Calpodes* fat body from esterase demonstration by Deimling's method. Scale 1 μ m.

ments provide strong evidence that the cap localisation of dehydrogenases is an artefact of Nitro B.T. formazan's attraction for lipid-water interfaces. This attraction has been observed in histochemical practice⁶ and has been demonstrated recently *in vitro*¹⁰.

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¹ Deimling, O. v., and Madreither, H., *Histochemie*, **29**, 83-96 (1972).

² Deimling, O. v., and Madreiter, H., *Histochemie*, **29**, 83-96 (1972).

³ Holt, S. J., *Proc. R. Soc.*, **B142**, 160-169 (1954).

⁴ Holt, S. J., and Withers, R. F. J., *Proc. R. Soc.*, **B148**, 520-532 (1958).

⁵ Locke, M., *Tissue Cell*, **2**, 197-223 (1970).

⁶ Novikoff, A. B., Shin, W.-Y., and Drucker, J., *J. biophys. biochem. Cytol.*, **9**, 47-61 (1961).

⁷ Pearce, A. G. E., *Histochemistry, Theoretical and Applied*, **2** (Churchill Livingstone, London, 1972).

⁸ Subramoniam, T., *Acta histochem.*, **47**, 250-253 (1973).

⁹ Thomsen, E., *Z. Zellforsch.*, **75**, 281-300 (1966).

¹⁰ Warchol, von J. B., and Marzotko, D., *Acta histochem.*, **42**, 378-380 (1972).

¹¹ Wigglesworth, V. B., *Q. Jl microsc. Sci.*, **99**, 441-450 (1958).

¹² Wigglesworth, V. B., *Nature*, **210**, 759 (1966).

¹³ Wigglesworth, V. B., *The Principles of Insect Physiology*, seventh ed. (Chapman and Hall, London, 1972).

Genetic response to environmental heterogeneity

ONE of the major problems of contemporary population genetics is how to account for the large amount of genetic variation occurring in natural populations. Considerable controversy

exists between those people proposing that the variation is adaptively neutral, and those arguing that most of the variation is maintained by balancing selection. One of the processes of balancing selection postulated, is based on the idea that different genetic variants are favoured in different environmental niches. We report results from an experiment designed to test this hypothesis in which populations of *Drosophila* were exposed to experimental environments of various degrees of heterogeneity. The rationale is simple—if genetic variation is adaptively neutral, all populations should maintain about the same amount of genetic variation; if the 'environmental heterogeneity' hypothesis is correct, the degree of genetic variation in a population should be correlated with the degree of heterogeneity of its environment.

Eighteen population cages were started on August 20, 1972, each consisting of 423 *Drosophila pseudoobscura* that were F2 and F3 progenies of gravid females collected at McDonald Ranch, Napa County, California during May and June 1972. Two females and one male were introduced into each cage from each of 141 single female lines. The cages were similar to those described by Ayala¹ but each contained ten food cups. Four environmental factors were varied in the experiments: food, yeast, temperature and illumination (see Table 2 for the environmental treatments used). In four populations all four environmental factors were fixed for any one population. In the other 14 populations, one or more environmental factors were heterogeneous; that is, these populations were exposed to two kinds of food, and/or two kinds of yeast, and so on. The number of environmental factors varied were: one in four populations, two in five populations, three in two populations, and four in three populations. The cages were maintained for 1 yr (12-15 generations) at which time adult females were collected and assayed for genetic variability using the techniques of starch-gel electrophoresis². Twenty polymorphic enzyme loci were studied in all populations except for three cages which we had sampled for only 15 loci before they were destroyed accidentally. Forty-four individuals or 88 genomes from each population were assayed for each enzyme. All cages were assayed within 3 weeks.

Table 1 summarises the results. For each group of populations we calculated the average (over populations) of heterozygous individuals observed at each locus. There is a tendency for higher levels of heterozygosity in populations exposed to greater environmental heterogeneity. The heterozygosity among the groups of populations is significantly heterogeneous at only two loci, *Ald* and *Est-5*.

Table 1 also shows the average heterozygosity over the 20 loci studied for each group of populations. The average heterozygosity gradually increases as the number of heterogeneous environmental factors increases from zero to three, but it decreases from three to four. Given the large standard errors, heterozygosities would not be expected to reflect precisely the environmental effects. Yet, the overall trend towards increased heterozygosity with increased environmental heterogeneity seems well established; the average heterozygosities are significantly heterogeneous. An analysis of variance based on the locus heterozygosities for all cages shows that treatment (number of environmental factors heterogeneous) has a significant effect ($P \approx 0.01$). The heterozygosity values for each population have also been calculated using only the 15 loci assayed in all populations. The results do not differ in any significant way from those reported in Table 1.

The average proportion of heterozygotes per locus in the natural population from which the experimental flies were descended is 0.212 ± 0.041 for the 20 polymorphic loci studied. This statistic averaged over all 18 experimental populations is 0.184 ± 0.006 . In the experimental populations the amount of genetic variation has decreased somewhat on the average, although for some groups of populations the average heterozygosity is ostensibly very similar to that of the natural populations.

Table 2 gives for each environmental factor the heterozy-

Table 1 Proportion of heterozygous loci per individual in 18 experimental populations of *Drosophila pseudoobscura*

Loci	No. of heterogeneous environmental factors				
	0	1	2	3	4
<i>Acph-1</i>	0.080 ± 0.072	0.062 ± 0.011	0.158 ± 0.034	0.112 ± 0.021	0.092 ± 0.023
<i>Acph-2</i>	0.153 ± 0.022	0.170 ± 0.054	0.134 ± 0.020	0.136 ± 0.023	0.151 ± 0.059
<i>Ald</i> †	0.165 ± 0.022	0.098 ± 0.008	0.170 ± 0.019	0.295†	0.242 ± 0.033
<i>Aph-1</i>	0.193 ± 0.034	0.227 ± 0.031	0.273 ± 0.040	0.273 ± 0.000	0.235 ± 0.072
<i>Aph-2</i>	0.107 ± 0.019	0.119 ± 0.023	0.145 ± 0.026	0.193 ± 0.011	0.091 ± 0.023
<i>Est-4</i>	0.040 ± 0.019	0.074 ± 0.014	0.050 ± 0.018	0.125 ± 0.057	0.023 ± 0.013
<i>Est-5</i> §	0.341 ± 0.089	0.528 ± 0.035	0.591 ± 0.047	0.528 ± 0.063	0.568 ± 0.035
<i>G3pdh</i>	0.080 ± 0.015	0.091 ± 0.052	0.148 ± 0.022	0.136†	0.114 ± 0.023
<i>Hk-1</i>	0.074 ± 0.044	0.102 ± 0.035	0.052 ± 0.021	0.068 ± 0.000	0.063 ± 0.038
<i>Hk-4</i>	0.040 ± 0.017	0.098 ± 0.008	0.091 ± 0.023	0.023 ± 0.023	0.045 ± 0.026
<i>Idh</i>	0.000 ± 0.000	0.000 ± 0.000	0.014 ± 0.009	0.011 ± 0.011	0.000 ± 0.000
<i>Lap</i>	0.146 ± 0.058	0.250 ± 0.093	0.250 ± 0.025	0.223 ± 0.073	0.280 ± 0.059
<i>Me-1</i>	0.222 ± 0.049	0.290 ± 0.043	0.336 ± 0.032	0.384 ± 0.021	0.364 ± 0.013
<i>Me-2</i>	0.165 ± 0.023	0.176 ± 0.025	0.186 ± 0.023	0.159 ± 0.000	0.136 ± 0.013
<i>Mdh</i>	0.091 ± 0.076	0.061 ± 0.061	0.195 ± 0.077	0.000 ± 0.000	0.030 ± 0.030
<i>Odh</i>	0.136 ± 0.044	0.091 ± 0.023	0.068 ± 0.054	0.227†	0.068 ± 0.039
<i>Pgm</i>	0.318 ± 0.132	0.398 ± 0.031	0.472 ± 0.091	0.750 ± 0.114	0.470 ± 0.027
<i>Tpi-1</i>	0.017 ± 0.017	0.011 ± 0.011	0.018 ± 0.018	0.000 ± 0.000	0.000 ± 0.000
<i>Tpi-2</i>	0.023 ± 0.009	0.228 ± 0.134	0.045 ± 0.034	0.023 ± 0.023	0.038 ± 0.027
<i>Xdh</i>	0.523 ± 0.091	0.652 ± 0.053	0.631 ± 0.057	0.500†	0.689 ± 0.087
Average*					
20 loci	0.146 ± 0.029	0.186 ± 0.038	0.201 ± 0.040	0.208 ± 0.045	0.186 ± 0.044
17 loci	0.159 ± 0.032	0.193 ± 0.044	0.217 ± 0.046	0.224 ± 0.052	0.204 ± 0.051

* Averages have been calculated for all 20 loci sampled, as well as separately for 17 loci not located on the third chromosome. Analyses of variance using the interaction term as error show significant heterogeneity among the cages with different numbers of heterogeneous factors, $P \leq 0.01$.

† Only one cage sampled.

‡ Analysis of variance shows significant heterogeneity among the cages with different numbers of heterogeneous factors (classes 3 and 4 have been combined), $P < 0.01$.

§ $P < 0.005$.

gosities in populations exposed to each of two environments or to both. The values in Table 2 were obtained by averaging the average heterozygosities of the appropriate experimental populations. For every factor, populations exposed to heterogeneous environments have, on the average, greater amounts of genetic variation than populations exposed to uniform environments. The differences are highly significant for temperature and yeast, less so for food, and are statistically insignificant for light.

The idea that genetic polymorphism can be maintained by selection favouring two or more phenotypes in the same population is not new^{3,4}. Indeed, mathematical treatments of the subject have existed for 20 yr (refs 5 and 8). In spite of this early theoretical interest, field and laboratory data in support of multiple-niche polymorphism are far from extensive. The early work of Cain and Sheppard⁹ gave some indication that shell colour in *Cepaea* was related to the variety of habitats in which it was found. Likewise Da Cunha *et al.*¹⁰ presented evidence for a positive correlation between the frequency of gene arrangements in *D. willistoni* and the degree of habitat diversity. Laboratory experiments to demonstrate that polymorphism can be maintained by diversifying selection were carried out by Thoday and Boam¹¹ and Clarke and Sheppard¹² on the level of quantitative characters in *D. melanogaster* and *P. dardanus* respectively, and by Beardmore and his colleagues¹³ on the *esterase-6* locus in *D. melanogaster*. More recently Powell¹⁴ has reported the results of an experiment carried out in population cages with *D. willistoni*. Having an experimental design similar

to our own, Powell surveyed 13 populations for 22 enzyme loci and found that the average heterozygosity per individual was higher in populations maintained in heterogeneous environments than in populations in more constant environments. Since *D. willistoni* flies are polymorphic for multiple chromosomal inversions, however, it has been argued that the inversion polymorphisms and not the single loci may have been the direct targets of balancing selection in Powell's experiment¹⁵. This ambiguity does not arise in our experiment. The strains of *D. pseudoobscura* used to initiate our populations carry inversions only in the third chromosomes. Seventeen of the loci assayed in our study are located in chromosomes other than the third. The heterozygosities calculated using only these 17 loci are given in Table 1. Clearly, populations exposed to heterogeneous environments maintained more genetic variation than populations living in uniform environments. The parameters given in Table 2 have also been calculated for the 17 loci known to be in chromosomes other than the third. The results do not differ in any significant way from those obtained when all 20 loci are used.

Since our experiment was designed to test a model of selection we have taken care to minimise the role of random drift. Drift could have been a significant factor on the population heterozygosities if the population size were small at the start or at any time during the experiment. Each cage was started with 846 genomes (2×423 flies) descended from 282 wild flies (141 females and their mates); the number of flies in a cage was never smaller than 500.

In conclusion, our results indicate an overall positive correlation between environmental diversity and genetic polymorphism, and therefore support the notion that genetic variation is maintained in natural populations by selection.

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¹ Ayala, F. J., *Drosophila Information Serv.*, **43**, 185 (1968).

Table 2 Average heterozygosities for populations exposed to a given treatment

Factor	1	2	1/2	<i>t</i> *
Food	0.181 ± 0.021	0.173 ± 0.014	0.192 ± 0.004	1.90†
Yeast	0.176 ± 0.010	0.161 ± 0.012	0.194 ± 0.004	2.23‡
Temperature	0.168 ± 0.012	0.176 ± 0.012	0.207 ± 0.006	4.73§
Light	0.168 ± 0.012	0.192 ± 0.011	0.193 ± 0.007	1.38

1, Spassky's food, baker's yeast, 16° C, dark; 2, cornmeal medium, brewer's yeast, 25° C, light; 1/2, both 1 and 2 present.

* One-sided Student's *t* test calculated on the assumption of same variance for all three groups of populations within a given treatment.

† $P < 0.050$.

‡ $P < 0.025$.

§ $P < 0.005$.

- ² Ayala, F. J., Powell, J. R., Tracey, M. L., Mourão, C. A., and Pérez-Salas, S., *Genetics* 70, 113-139 (1972).
- ³ Dobzhansky, Th., *Genetics and the origin of species* (Columbia University Press, New York, 1951).
- ⁴ Mather, K., *Evolution* 9, 52-61 (1955).
- ⁵ Ludwig, W., *Zool. Anzeiger* (Ncne Ergebnisse und Probleme der Zoologie) 145, 516-537 (1950).
- ⁶ Levene, H., *Am. Naturalist*, 87, 331-333 (1953).
- ⁷ Li, C. C., *Am. Naturalist*, 89, 281-296 (1955).
- ⁸ Dempster, E. R., *Symp. quant. Biol.*, 20, 25-32 (1955).
- ⁹ Cain, A. J., and Sheppard, P. M., *Am. Naturalist*, 88, 321-326 (1954).
- ¹⁰ Da Cunha, A. B., Burla, B. H., and Dobzhansky, Th., *Evolution*, 4, 212-235 (1950).
- ¹¹ Thoday, J. M., and Boam, T. B., *Heredity*, 13, 205-218 (1959).
- ¹² Clarke, C. A., and Sheppard, P. M., *Heredity*, 14, 163-173 (1960).
- ¹³ Beardmore, J., in *Essays in honor of Theodosius Dobzhansky*, 299-313 (Appleton-Century-Crofts, New York, 1970).
- ¹⁴ Powell, J. R., *Science*, 174, 1035-1036 (1971).
- ¹⁵ King, J. L., *Science*, 176, 545 (1972).

Inferred slow inward current in snail neurones

A NUMBER of excitable membranes exhibit a long-lasting depression of the delayed outward current during a depolarising pulse. One explanation is that there is a true inactivation of potassium gates¹⁻³, and another is that it is due to an accumulation of potassium ions in a limited volume extracellular space, reducing the electric driving force for potassium ions^{3,4}. We have examined the relationship between the depression of net outward current and concomitant changes in potassium ion activity recorded with a potassium-sensitive microelectrode positioned close to the surface of monopolar ganglion cells of the snail *Helix pomatia*.

Experiments were performed on two bursting pacemaker neurones in the right parietal ganglion⁵. One of these produces no fast outward current⁶⁻⁹ and is therefore useful as a control for possible artefacts due to activation or inactivation of an additional outward current. Electrodes of 2-20 M Ω , filled with 3 M KCl, were inserted into exposed cell bodies. The preparation was bathed in Ringer's solution¹⁰ with 5 mM HEPES (N-2-hydroxyethyl-piperazine-2-ethane sulphonic acid; Servo, Heidelberg) buffer in place of Tris at a temperature of 13°C-15°C. The electrochemical activity of K⁺ was monitored at distances below 50 μ m from the visible cell surface with a double-barrelled potassium sensing electrode. One barrel contained the K⁺ selective resin while the other barrel, containing 0.15 M NaCl, acted as a reference for the pair¹¹. The resolution time constant for transients in K⁺ activity was below 20 ms. Membrane current during voltage clamping of the cell was measured with glass pipettes from a limited area of the soma surface (50-80 μ m in diameter) by the patch clamp technique¹². This avoided current transients from unclamped regions of the neurone. All signals were stored on tape and averaged by a Didac computer. Net charge transfer across the membrane (integral of the current signal) was compared with net K⁺ flux (time integral of the K⁺-activity signal).

The major features characterising depression of delayed outward current can be seen in Fig. 1a. Long depolarising pulses produced a delayed outward current which gradually decayed to about 60% or less of its maximum intensity with a time constant of 300 to 700 ms (refs 1, 7). The presentation of paired short pulses of equal amplitude and duration demonstrated that long term depression of the slow outward current persisted even though the voltage was returned to the holding level between a conditioning pulse (pulse 1) and a test pulse (pulse 2). For a time interval of up to 1 s, the delayed current produced by the second pulse sometimes even failed to reach the level of the current produced at the same point in time by the prolonged first pulse. Recovery from depression of the pulse 2 outward current occurred with a time constant of up to 10 s. The length of pulse 1 necessary to depress the test pulse current corresponded with the rise of the pulse 1 outward current. Prolongation of pulse 1 beyond its peak did not produce much further depression

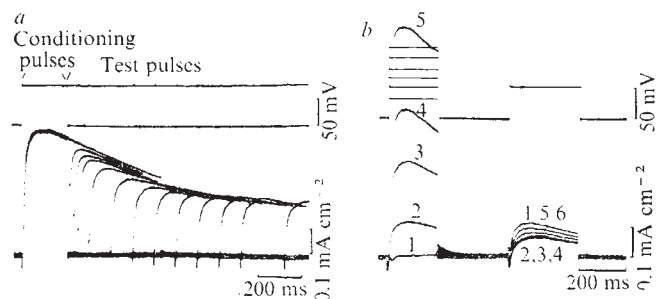


Fig. 1 a, Superimposed series of a conditioning, test pulse experiment. Voltage programme, at the top (holding potential -58 mV), current traces below. Pulse 1 is held at a fixed position while pulse 2 is delivered with various delays to show the envelop of the outward current depression. A single pulse of equal voltage but longer duration (650 ms) is also superimposed. b, Depression of pulse 2 current by increasing amplitude of pulse 1. Same cell and holding potential as in a. Small numbers identify corresponding sequences. Pulse 2 current slopes numbered 5 and 6 rise from considerably higher instantaneous currents as an aftereffect of the large pulse 1 currents. The pulse 1 current during the 120 mV step⁶ is not displayed.

of the pulse 2 current¹. This, together with the failure of sub-threshold conditioning pulses to produce the phenomenon, suggests that the activation of the current gates during pulse 1 may be causally related to the time-dependent depression of the pulse 2 outward current. Increasing pulse 1 further reduced pulse 2 currents, but only slightly (Fig. 1b). The maximum effect was reached at positive membrane voltages of pulse 1 (+40 to +100 mV).

The kinetics of closing of the activated gates at the termination of pulse 1 can be determined from the instantaneous current steps which appeared at the onset of subsequent pulses 2 at short intervals (Fig. 1a). The depression of pulse 2 current was much longer-lasting and must therefore have arisen by a different mechanism. In bursting pacemaker cells, the rising phase of pulse 2 outward currents is always considerably slower than that of pulse 1 (Fig. 2a, b), which may contribute to the failure of pulse 2 currents to reach the current level during maintained voltage steps (Fig. 1a). Although the pulse 2 outward current increased up to 30% if hyperpolarisation up to -150 mV preceded pulse 2, the retardation of the current remained and became more pronounced during the early part of the outward current trajectory.

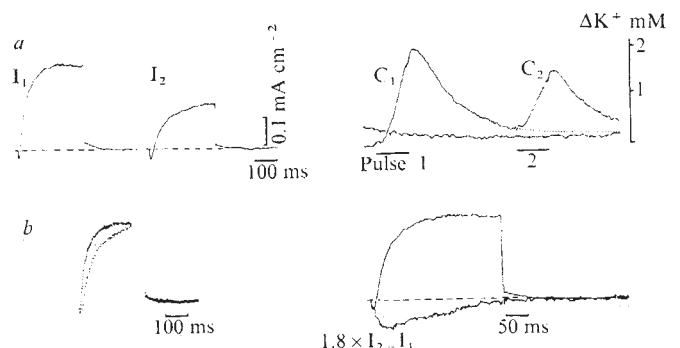


Fig. 2 a, Left, averaged pulse 1 and pulse 2 currents (I) ($n=8$) of a cell A during voltage steps of $+45$ mV from holding potential of -42 mV. The interpulse pause was 1 s. Right, simultaneously recorded extracellular K⁺ signals (C) in a continuous display. The tail K⁺ signal is superimposed. The prolonged slope from a sample of single pulses ($n=8$) at the same electrode position is marked by dots. Calibration of K⁺ signals is made with Ringer solutions with known K⁺ molarities¹¹. Note that current (left) and K⁺ signal (right) are displayed at different time scales. b, Left, currents normalised to same peak amplitude to show retardation in rising phase of pulse 2 current (digitalised display). Right, after normalisation, pulse 1 current subtracted from (enlarged) pulse 2 current; inward current downwards. Pulse 1 current is superimposed.

The long-lasting depression of outward current provided a convenient situation for comparing the depression of net outward current during pulse 2 with the concomitant reduction in K^+ accumulation at the outer neuronal surface using the K^+ -specific microelectrode (Figs 2–4). We standardised the pause between pulses 1 and 2 at 1,000 ms and delivered paired pulses at a repetition rate of once every 30 s to allow time for complete recovery from depression. The ratio $Q(2:1)$ was obtained by dividing the time integral of net outward current during pulse 2 by the equivalent integral during pulse 1 and was found to be 0.51 ± 0.12 (s.d.).

Since the outward current ceases almost immediately on termination of the pulse, accumulated extracellular K^+ (ΔK^+) should dissipate and its concentration fall progressively from the relatively early peak after the pulse is terminated. The role of K^+ accumulation was tested directly by measuring the K^+ activity with the electrode very near or just touching the visible cell surface during paired depolarising pulses. Single pulses were also applied to determine the baseline activity for the second pulse. After 1 s, when pulse 2 was initiated, the potassium signal evoked by pulse 1 was less than 15% of its peak value. Nevertheless, the outward current during pulse 2 was always depressed for periods longer than 1 s when the extracellular K^+ activity had approached even closer to pre-stimulus levels. Thus it seems unlikely that the depression of the pulse 2 net outward current is primarily due to extracellular accumulation of potassium ions.

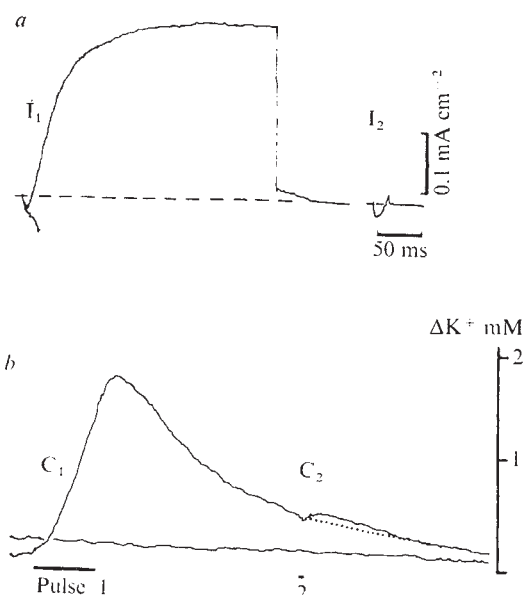


Fig. 3 Same experiment ($n=12$) and voltages as in Fig. 2, with pulse 2 of much shorter duration (20 ms). *a*, Current signal; *b*, K^+ activity signal. There is an obvious K^+ signal following pulse 2, concomitant with a predominantly inward going current during that pulse. Ratio of outward currents ($Q(2:1)$) is not proportional to the ratio of K^+ signals ($FK(2:1)$). Current and K^+ activity traces displayed at different time scales.

If it is assumed that the net outward current recorded under voltage clamp conditions is a pure potassium current, it follows that potassium activity changes recorded near the cell surface should parallel quantitatively the net charge transfer produced by the outward current. That is, the ratio $Q(2:1)$ should equal the ratio $FK(2:1)$ (K flux during pulse 2/ K flux during pulse 1) for any pair of pulses. From Figs 2 and 4 it is evident that $FK(2:1) < 1$; that is, K^+ efflux is reduced, as would be expected if the K channels are partially inactivated during pulse 2. Quantitative comparison, however, indicates that there is a 'deficit' in the net outward current of pulse 2 relative to the K^+ signal. In Fig. 2*a* this is obvious from the peak of the K^+ signal of pulse 2 which is more than half of the K^+ peak of pulse 1, whereas the ratio of the amplitudes of currents 2 and 1 is less than 0.5. This deficit ($FK(2:1) - Q(2:1)$) ranged from 0.10 to

0.25 in various units studied. The deficit in net current is most readily explained as the result of an inward current which makes its appearance during pulse 2 and shortcircuits a portion of the K^+ efflux. Such an inward current would result in a net charge transfer during pulse 2 lower than that transferred by K^+ efflux, and thus explain the deficit of net outward current recorded during pulse 2 relative to the K^+ signal.

The presence of a previously unrecognised slow inward current receives further support from a comparison of the kinetics of the outward currents of pulses 1 and 2 (Fig. 2*a, b*). The trajectory of the outward current characteristically has a slower rising phase during pulse 2 than during pulse 1 when the currents were normalised. (Normalisation was carried out by three methods. In the first, the pulse 2 current was simply additionally amplified to bring the current peaks of the two pulses to the same amplitude. In the second, the exponential falling phases of the current pulses were extrapolated back to the beginnings of the pulses and the pulse 2 current was then additionally amplified, so that the extrapolated decays began from the same amplitude. In the third method of normalisation the averaged pulse 1 current trajectory was reduced in proportion to the ratio $FK(1:2)$ and then subtracted from the averaged trajectory of pulse 2. Deficit currents determined by those three methods differed only slightly.) Comparison of current traces in Fig. 2*a* and 2*b* shows that the 'slow inward current' obtained by subtracting normalised pulses 1 from pulses 2 has a far slower time course than the fast inward current.

The difference in the trajectory of the outward current of pulses 1 and 2 was displayed by electronically subtracting, after normalisation, the averaged trajectories of pulse 1 currents from those of the enlarged pulse 2 currents from the same neurone (Fig. 2*b*). The result is a trajectory with a peak 20 to 50 ms after the onset of the current pulse, that is, during the rising phase of the outward current of pulse 1. The time integral of this deficit in pulse 2 current in the different cells was -0.08 to -0.21 times that of the corresponding pulse 1 currents and was always found comparable with the deficit in net outward current obtained by determining $FK(2:1) - Q(2:1)$.

We conclude that the long-term suppression of net outward current by a conditioning pulse consists of two components. The chief component is a true time and voltage-dependent inactivation of the delayed potassium system, similar in some respects to the inactivation of the sodium system described by Hodgkin and Huxley¹³. It is also manifested as a reduction in pulse 2 extracellular potassium accumulation relative to accumulation during pulse 1. Since it develops during interpulse periods with pulse durations approximating the duration of the action potential (Fig. 4), the inactivation contributing to the depression of outward current described here seems more likely to have functional importance under physiological conditions than any reduction in outward current which may be produced by the extracellular accumulation of K^+ with prolonged depolarising pulses⁴. The second component contributing to depression of delayed outward current during pulse 2 seems to be a previously unrecognised inward current which partially shortcircuits the outward potassium current. Its existence is supported by several independent lines of evidence: first, the reduction in K^+ efflux during pulse 2, resulting from a true K^+ inactivation, is smaller than the reduction in net outward current (Fig. 2*a*); second, at any given time after pulse 1 (up to 1 or 2 s) the peak outward current produced by pulse 2 does not reach the level of outward current produced at that time by the prolongation of pulse 1 (Fig. 1*a*). In addition, the rising phase of pulse 2 exhibits slower kinetics than that of pulse 1, consistent with a superimposed delayed inward current (Fig. 2*b*).

Certain molluscan neurones have a fast outward potassium current which is activated by conditioning hyperpolarisation and inactivated by depolarisation⁶⁻⁹. Inactivation of a fast current might produce the observed slowing of the pulse 2 outward current. This source of artefact can, however, be ruled out for several reasons, the most important of which is the use of bursting pacemaker cells (Figs 1–3) which fail to show a fast out-

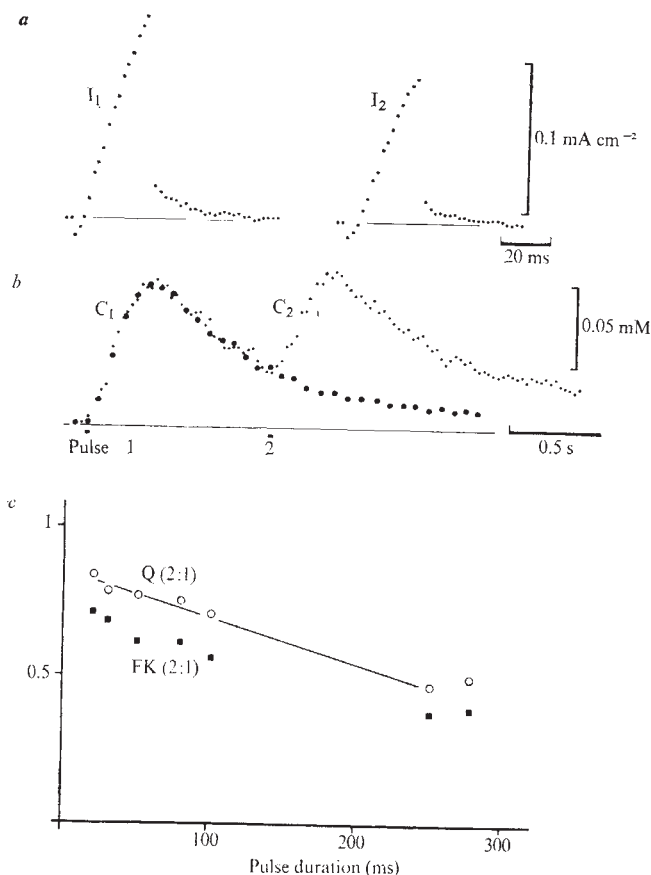


Fig. 4 *a*, Currents 1 and 2 evoked by +65 mV steps each of 30 ms duration from a holding potential of -45 mV. Simultaneous sampling of K⁺ signals is shown in *b* ($n=25$). The superimposed averaged trajectory of single pulses is given by heavier dots. *c*, Ratios of pulse 2 to pulse 1 outward current integrals ($Q(2:1)$) and corresponding ratios of potassium fluxes ($FK(2:1)$) of this cell plotted as a function of pulse duration. Duration of pulse 1 and 2 was kept equal and was varied in unison. At shorter pulse durations the difference between K⁺ flux and charge transfer ($FK(2:1) - Q(2:1)$) becomes smaller, but does not disappear.

ward current even if given a strong hyperpolarising conditioning pulse. Also, the deficit of pulse 2 outward current relative to measured K⁺ accumulation cannot be explained by the inactivation of a fast outward current. In cells with a fast outward current this deficit was seen whether or not the fast outward current was activated.

The delayed inward current is difficult to identify and measure during pulse 1; its appearance or augmentation during pulse 2 has been demonstrated by using the pulse 1 current as a control for the pulse 2 current. It is nevertheless interesting that the activation of this inward current, unlike the other membrane current components, is facilitated by a preceding depolarisation. The two components of delayed outward current depression, true inactivation of the K⁺ system and activation of a slow inward current, presumably both contribute to the progressive increase in spike duration, overshoot and positive shift in post-spike undershoot, which occur during the initial half dozen or so spikes of the train comprising a 'burst'. The ionic identity of the slow inward current and its possible role in neuronal bursting remain to be ascertained.

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- ¹ Lux, H. D., and Neher, E., *Proc. fourth Int. Congr. Biophys.*, **3**, 222-223 (1972).
- ² Gola, M., *Pflügers Arch., ges. Physiol.*, **346**, 121-140 (1974).
- ³ Alving, B. O., *J. gen. Physiol.*, **54**, 512-531 (1969).
- ⁴ Eaton, D. C., *J. Physiol., Lond.*, **224**, 421-440 (1972).
- ⁵ Kerkut, G. A., and Meech, R. W., *Comp. Biochem. Physiol.*, **19**, 819-832 (1966).
- ⁶ Hagiwara, S., and Saito, N., *J. Physiol., Lond.*, **148**, 161-170 (1959).
- ⁷ Neher, E., and Lux, H. D., *Pflügers Arch. ges. Physiol.*, **322**, 35-38 (1971).
- ⁸ Neher, E., *J. gen. Physiol.*, **58**, 36-53 (1971).
- ⁹ Connor, J. A., and Stevens, C. F., *J. Physiol., Lond.*, **213**, 31-53 (1971).
- ¹⁰ Gainet, H., *Brain Res.*, **39**, 369-385 (1972).
- ¹¹ Lux, H. D., and Neher, E., *Expl Brain Res.*, **17**, 190-205 (1973).
- ¹² Neher, E., and Lux, H. D., *Pflügers Arch. ges. Physiol.*, **311**, 272-277 (1969).
- ¹³ Hodgkin, A. L., and Huxley, A. F., *J. Physiol., Lond.*, **117**, 500-544 (1972).

Neuromuscular blocking action of an alkylating local anaesthetic: site of action and effects of temperature and calcium ions

THE mechanism by which local anaesthetics block neuromuscular transmission has been under investigation for many years. Most of the evidence suggests that these drugs act primarily on nerve terminals either to inhibit acetylcholine (ACh) release¹⁻³ or exert some other effects⁴. However, other workers have shown inhibition of ACh receptor^{5,6} or direct depression of muscle excitability⁷⁻¹⁰. Inhibition of nerve conduction apparently has been ruled out as contributing to the blockade in rat phrenic nerve-diaphragm⁷ or cat soleus nerve-muscle preparations⁸.

In an attempt to determine the primary site of action of these drugs we have used the recently synthesised β -haloethyl amine compound, 2-[(2-chloroethyl) methylamino] ethyl 4-ethoxybenzoate, which irreversibly blocks nerve conduction¹¹ as well as neuromuscular transmission¹². We report here that when phrenic nerve-diaphragm transmission is irreversibly blocked, the compound has essentially no effect on the excitability of nerve or muscle. From this we conclude that the action of the irreversible local anaesthetic in this preparation is confined to the junctional region.

The rat phrenic nerve-diaphragm preparation was set up by conventional means¹³ for stimulation of the muscle either directly or through the nerve (indirect). Figure 1 shows the effect of the compound on contractions elicited by both types of stimulation: the compound blocked indirect contractions with no effect on directly elicited contractions. This occurred whether the drug was in the bath or removed by washout. When the phrenic nerve was removed at the time of irreversible block of indirect contractions, it still maintained excitation and conduction properties. Irreversible block of these electrical responses *in vitro* required a concentration of drug about five times greater than that needed for irreversible block of transmission¹¹.

These findings show that blockade of transmission by the compound is confined to the junction. The physicochemical properties of the compound probably account for this selective action, unlike procaine which depresses both direct and indirect contractions concomitantly. At the pH of the bath we used (7.4) the compound exists almost exclusively in the aziridinium or positively charged form¹² and thus cannot penetrate well to the active membranes of nerve or muscle. On the other hand, the compound evidently can readily reach the unmyelinated nerve terminal membrane or post-junctional region, where it combines irreversibly with some receptor.

If the drug was washed out after a fairly short time of contact with the tissue, for example, 10 min, the observed

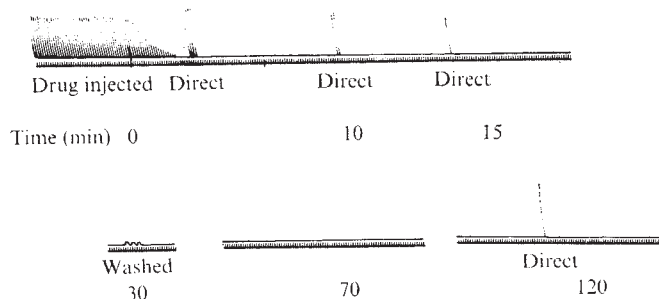


Fig. 1 Neuromuscular blocking action of 2-[(2-chloroethyl)methylamino]ethyl 4-ethoxybenzoate, 0.2 mg ml⁻¹, applied to rat phrenic nerve-diaphragm preparation. Even in the presence of the drug, the directly elicited contraction was unaffected. On the other hand, the indirect twitch was abolished. The irreversible nature of the latter effect is indicated by the complete block even 2 h after washing.

block of contraction was reversed completely, indicating a two step inhibitory mechanism similar to that observed with other receptor alkylating agents¹⁴.

If the tissue was pretreated with decamethonium or *d*-tubocurarine at concentrations sufficient to block transmission completely, application of the compound still resulted in irreversible blockade after washout. This procedure has been shown¹⁵⁻¹⁷ to protect the active site on the ACh receptor against irreversible reaction with α -bungarotoxin. Based on this finding, we think it most likely that a receptor for local anaesthetics exists on the nerve terminal and that it can undergo the same type of alkylation reaction exhibited by receptors for neurohormones.

To obtain thermodynamic data for reaction with the receptor, the temperature dependence of rate of irreversible block of transmission was determined as follows. Before the drug was added, the nerve was stimulated with gradually increasing voltage, from threshold to the maximum for eliciting contraction of the muscle. This gave control voltage-response curves. This procedure was repeated at various times after the drug had been added to the bath and washed out, giving data for the irreversible change in

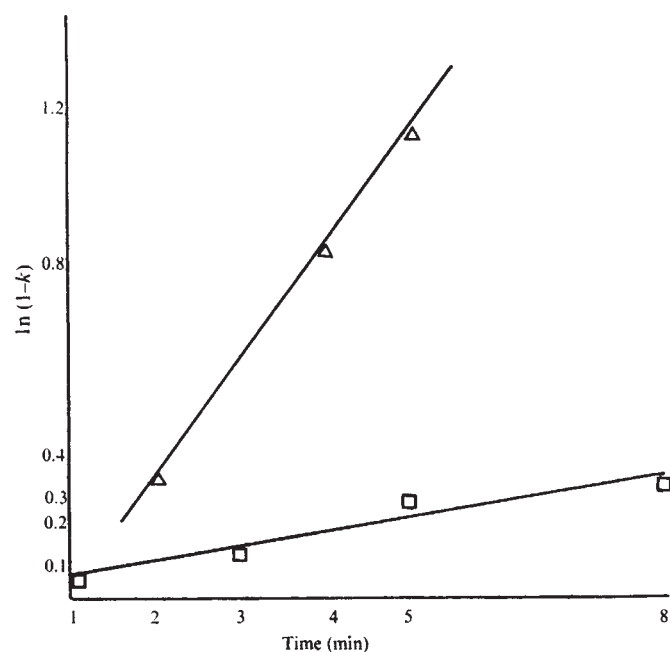


Fig. 2 Semi-log plot of rate of irreversible block of indirectly elicited twitch of the phrenic-nerve diaphragm by 2-[(2-chloroethyl)methylamino]ethyl 4-ethoxybenzoate. Rate of block was determined from midpoints of the voltage-response curve after washing out the drug (see text for details). □, k at 30° C = 0.037 min⁻¹; △, k at 15° C = 0.30 min⁻¹.

sensitivity. The initial effect of the drug was to shift the voltage-response curve to the right without reducing the maximum; with increasing time, the maximum declined and ultimately contractions could not be elicited at any voltage. The midpoints of the curves were plotted as log percentage change in voltage against time; straight lines were obtained (Fig. 2).

It is interesting that the rate constant at 15° C was much greater than at 30° C; this was not anticipated since alkylation reactions would be expected to show a positive temperature dependence. Other findings indicated that the actual rate of reaction of the drug with the receptor is much lower at the low than the high temperature. If the tissue was incubated with the drug at 15° C to give complete irreversible block and the temperature raised to 30° C, transmission was completely restored (Fig. 3). This indicates that during the time of reaction at 15° C only a small fraction of the receptors had reacted, but this was sufficient to produce the complete block at this temperature. Another possibility is that reversal of block was due to cleavage of the drug-receptor bond at the higher temperature. To test this, the degree of reversal was determined when the temperature was progressively raised from 15° to 30° C and then lowered back to 15° (Fig. 4). The two curves are almost identical and thus the reversal of the block produced by raising the temperature was not due to a change in receptor occupancy. In addition, if the drug was applied at 30° C for a time during which no significant irreversible shift in the voltage-response curve was observed; lowering the temperature to 15° C resulted in inhibition of contractions.

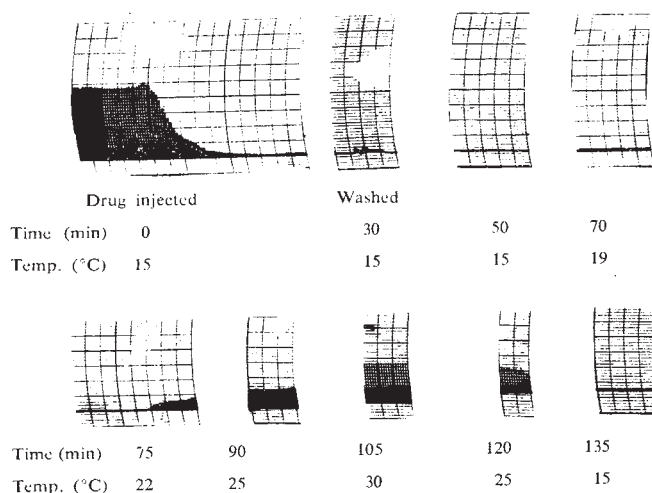


Fig. 3 Effect of increasing temperature of bath after application and washout of 2-[(2-chloroethyl)methylamino]ethyl 4-ethoxybenzoate, 0.2 mg ml⁻¹, for 30 min. The indirect twitch was irreversibly blocked at 15° C and progressive increase in temperature resulted in relief of block. At 30° C the response was almost that of the 30° C control, which usually was somewhat smaller than at 15° C. Reduction of the temperature back to 15° C resulted in return of the block.

These results indicate that the number of receptors reacting irreversibly is directly related to temperature whereas the rate of block is inversely temperature dependent. Thus the rate of receptor alkylation cannot be determined pharmacologically since there appears to be no direct relationship between absolute number of receptors occupied and the degree of neuromuscular block. This was shown by the fact that although the same number of receptors was occupied by the drug at 15° and 30° C, the extent of the block differed. One possible explanation for the faster blockade at the lower temperature in spite of a smaller fraction of receptors occupied is that fewer nerve elements are involved in transmission at the lower temperature.

Hence, occupation of a smaller fraction of nerve terminal receptors would be sufficient to block contractions. In support of this concept, Taylor reported that the output of acetylcholine is about 80% lower at 20°C as compared with 37°C in this preparation¹⁸.

It is well documented that curare-like drugs also show an inverse temperature dependence for potency of neuromuscular blockade^{18,19}. This has been interpreted in terms of a temperature-dependent dissociation of the drugs from the ACh receptor¹⁹ or to temperature dependent solubility

of the drugs in the post-synaptic membrane²⁰. We have found that blockade of transmission by procaine shows a similar inverse temperature dependence. In view of the results reported here for the irreversibly acting compound, the explanation for temperature dependence of blockade by reversibly acting drugs may have to be revised.

The effects of calcium on the voltage-response curve of the alkylating compound and on procaine are shown in Fig. 5. In this concentration range, calcium had no effect on excitability of the preparation. Calcium ions had a biphasic effect on the irreversible compound, promoting its potency at low concentrations, inhibiting its blocking action at higher concentrations. On the other hand, calcium only inhibited procaine blockade of transmission. The potency of the alkylating compound was also enhanced when the preparation was stimulated tetanically or if the potassium ion concentration was increased. These conditions produce nerve terminal depolarisation. Since the compound was present primarily in the quaternary ammonium form, it would not penetrate well through the nerve terminal membrane. Penetration seems to have been promoted by depolarising conditions as manifested by the enhanced potency observed. These results are in harmony with Narahashi's evidence that the site of action of local anaesthetics is the inner surface of nerve axon membranes²¹. Higher concentrations of calcium ions probably reduce the rate of irreversible reaction of the aziridinium compound with its receptor by a competitive mechanism as observed on various nerve axons for reversible local anaesthetics^{22,23}.

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- ¹ Straughan, D. W., *J. Pharmacol.*, **13**, 49 (1961).
- ² Matthews, E. K., and Quilliam, J. P., *Br. J. Pharmacol.*, **22**, 415 (1964).
- ³ Galindo, A., *J. Pharmacol. exp. Ther.*, **177**, 360 (1971).
- ⁴ Usubiaga, J. E., and Standaert, F. G., *J. Pharmacol. exp. Ther.*, **159**, 353 (1968).
- ⁵ Del Castillo, J., and Katz, B., *Proc. R. Soc.*, **B146**, 339 (1957).
- ⁶ Maeno, T., *J. Physiol., Lond.*, **183**, 592 (1966).
- ⁷ Hirst, G. D. S., and Wood, D. R., *Br. J. Pharmacol.*, **41**, 105 (1971).
- ⁸ Inoue, F., and Frank, G. B., *J. Pharmacol. exp. Ther.*, **136**, 190 (1962).
- ⁹ Lorkovic, H., *Arch. int. Pharmacodyn.*, **146**, 266 (1963).
- ¹⁰ Harris, E. J., and Ochs, S., *J. Physiol., Lond.*, **187**, 5 (1966).
- ¹¹ Rosen, G. M., Ehrenpreis, S., Mittag, T. W., and Stubbins, J. F., *J. med. Chem.*, **14**, 514 (1970).
- ¹² Rosen, G. M., Ehrenpreis, S., and Karoutsou, A., *Arch. int. Pharmacodyn.*, **204**, 242 (1973).
- ¹³ Bulbring, E., *Br. J. Pharmacol.*, **1**, 38 (1946).
- ¹⁴ Nickerson, M., and Gump, W. S., *J. Pharmacol. exp. Ther.*, **97**, 25 (1949).
- ¹⁵ Chang, C. C., and Lee, C. Y., *Arch. int. Pharmacodyn.*, **144**, 241 (1963).
- ¹⁶ Changeaux, J.-P., Kasai, M., and Lee, C. Y., *Proc. natn. Acad. Sci. U.S.A.*, **67**, 1241 (1970).
- ¹⁷ Miledi, R., and Potter, L. T., *Nature*, **233**, 599 (1971).
- ¹⁸ Taylor, D. B., *Anesthesiology*, **20**, 439 (1959).
- ¹⁹ Lonsdale, K., and Milledge, H. J., *Nature*, **206**, 407 (1965).
- ²⁰ Canepa, F. G., *Nature*, **207**, 1149 (1965).
- ²¹ Narahashi, T., and Frazier, D. T., in *Neurosciences Res.* (edit. by Ehrenpreis, S., and Solnitzy, O. C.), **4**, 66 (Academic Press, New York, 1971).
- ²² Blaustein, M. P., and Goldman, D. E., *J. gen. Physiol.*, **49**, 1043 (1966).
- ²³ Sasa, M., Avner, B. P., and Albuquerque, E. X., *Eur. J. Pharmacol.*, **23**, 97 (1973).

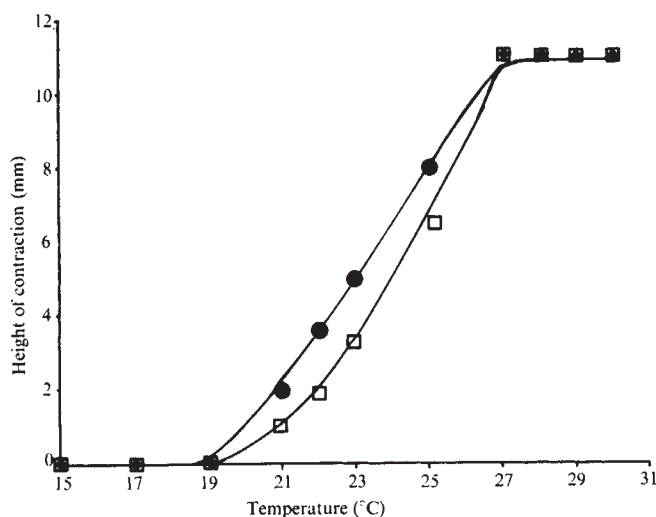


Fig. 4 Effect of progressive increases of temperature from 15°C to 30°C (□) and the subsequent return to 15°C (●) on a preparation blocked irreversibly at 15°C. The degree of irreversible block was not significantly affected by the increase in temperature, and thus the appearance of contractions did not result from dealkylation of receptors.

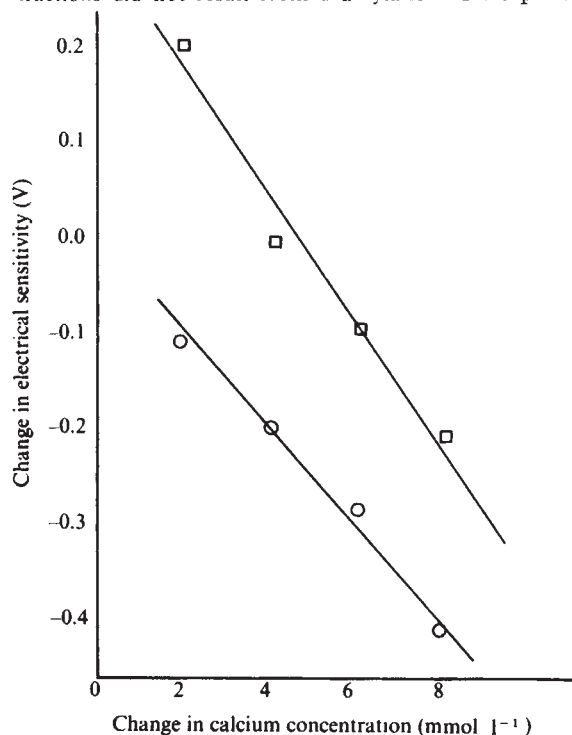


Fig. 5 Effect of increasing calcium ion concentration on change in sensitivity of indirectly elicited contraction by the alkylating local anaesthetic (□) and by procaine (○). Sensitivity (V) is the voltage required to produce half-maximum contraction as determined after applying the drugs for 10 min. An increase in ΔV indicates that the calcium increased the potency of the drug, that is a greater voltage was required to produce half-maximum contraction. A decrease in ΔV signifies antagonism of the drug by calcium.

Temperature-sensitive mutation affecting myofilament assembly in *Caenorhabditis elegans*

THE assembly of contractile and calcium-related regulatory proteins into functioning myofilament arrays within muscle cells and the genetic control of muscle differentiation are still largely unknown processes¹⁻². Here we present evidence that a particular gene may regulate such lattice assembly in the body wall muscle cells of the nematode *Caenorhabditis elegans*. A temperature-sensitive mutation in this gene can prevent the appearance of normal myofilament lattices in this muscle without affecting the amounts of major contractile proteins present. The organised or defective adult structures once formed in mutant muscle are stable to changes in temperature.

The recessive mutation *e286* is induced by ethyl methane-sulphonate on chromosome III of *Caenorhabditis elegans* (strain collection of Dr Sydney Brenner)^{4,5}. This mutagen has proved of great use in producing temperature-sensitive mutations in *Drosophila*⁶. *N*₂ (wild type) nematodes are highly motile between 15° and 25° C. The locomotive and reproductive rates of these animals increase over this temperature range. In contrast, when grown above 20° C, *E286* animals are paralysed with only very slow body movements apparent and they reproduce at a diminished rate. At 15° C, the movement and growth of *E286* mutants is indistinguishable from *N*₂ populations. At both temperatures, the pharyngeal muscle in mutants behaves normally. *+e286* heterozygotes are not affected behaviourally or structurally by growth at either temperature. Thus, one genic dose of normal function is sufficient to overcome the presence of a mutant gene or its product.

If mutant eggs (chitin-encased embryos) are permitted to develop and hatch at 15° C, switching the temperature to 25° C will lead to paralysed larvae and adults. Conversely, mutant embryos hatching at 25° C will develop into motile adults by growth at 15° C. Such reversal of phenotype can be performed throughout the four larval stages of these nematodes. Behavioural reversibility at the L₄ stage is demonstrated in Table 1. But when the sexually mature adult stage is reached, the phenotype is stable to temperature change within the above range. Maintenance of paralysed adults at 15° C or of motile adults at 25° C for periods up to several days (compare to 3 d generation time) does not alter either body movement, or the characteristic body wall muscle structure of either form (Fig. 1). Motile *E286* adults have body wall muscle cells exhibiting periodic sarcomeric structures such as A bands, I bands, dense bodies, and H zones that are very similar to *N*₂ muscle elements as observed with the polarised light microscope. Paralysed *E286* adults do not exhibit these regular structures in their body wall muscle. When the body wall muscle cells of *E286* animals grown at either temperature are examined by electron microscopy, ultra-structural differences between the two populations are observed. The 15° C specimen demonstrates thick filaments encircled by parallel arrays of thin filaments (Fig. 2). The paralysed 25° C animal also possesses recognisable thick filaments. In this case, there does not seem to be any specific orientation of thin filaments. Fibrous structures of dimensions appropriate to thin filaments appear at various angles to the thick filament array.

This mutation seems to be affecting the formation of myofilament arrays during muscle growth periods as evidenced by temperature reversibility of phenotype during larval development. Adult muscle having constructed its myofilament arrays or not is then unaffected by this temperature-sensitive mutant function. Thus, the instability of a permanent component of muscle is probably not responsible for the loss of structure and function at high temperature.

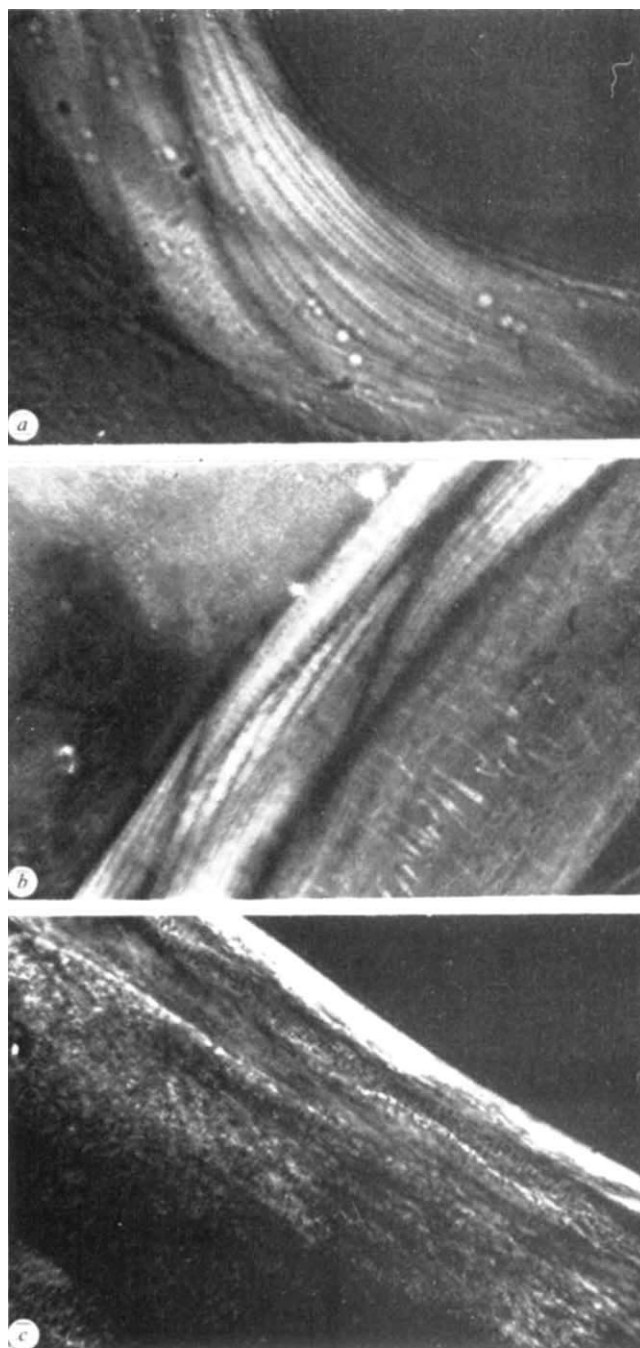


Fig. 1 Living animals were picked off lawns of *Escherichia coli*, OP50, and suspended in a 10 μ l drop of 0.8 *Ascaris* Ringer's solution (24.5 mM KCl, 11.8 mM CaCl₂, 9.8 mM MgCl₂, 3.9 mM NaCl, and 125.1 mM sodium acetate). A No. 1 or No. 1.5 glass coverslip was then placed over the drop. All observations were performed on a Reichert Zetopan research microscope fitted with strain-free objective and condenser. The uncalibrated magnification determined by instrumental and photographic settings was $\times 746$. *a*, *N*₂ grown at 25° C; *b*, *E286* grown at 15° C; *c*, *E286* grown at 25° C. All observations were made at an ambient temperature of about 23° C.

The relative migration and amounts of several myofilament proteins of *E286* grown at either temperature and of *N*₂ are similar to one another when separated according to molecular weight on sodium dodecyl sulphate-polyacrylamide gel slabs⁷. But a difference in a catalytic or minor structural protein present in low relative concentration or an alteration not affecting molecular weight would not be observed with this method.

Table 1 Reversibility of E286 locomotive behaviour

Temperature, condition* (°C)	No. of animals	Time (s) for 50 beats†
15	20	20.9 ± 1.8
25→15	20	31.2 ± 8.4
25	16	157 ± 74
15→25	16	189 ± 106

*Animals were either grown through adulthood at one temperature or switched at L₄ stage. All observations were of adults.

†One beat is defined as the formation of a semicircle by flexion at the mid-body when the animal is suspended in 0.8 Ascaris Ringer's solution. Note that about two thirds of the 25° C animals did not make any such movements and were not scored. The movements of 25° C and 15°→25° C animals that were motile at all were so slow that less than 50 beats were usually measured and then normalised to 50. Such biases may lead to underestimation of the time and greater variability in the paralysed cases.

What kind of function is altered by *e286*? The most attractive hypothesis is a defect in a catalytic function necessary for proper myofilament arrays to form, consistent with the fully recessive behavioural and morphological phenotype of E286 at 25° C. Mutation in a *cis* acting control element regulating synthesis of a myo-

fibrillar component would also explain the recessive character but would be unlikely to produce temperature sensitivity, whereas alteration of a structural protein which at 25° C prevented assembly might be expected to have a semi-dominant phenotype. Further biochemical and structural studies of E286 and the search for additional mutations within this gene are being pursued for better understanding of this function.

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- ¹ Etlinger, J. D., and Fischman, D. A., *Cold Spring Harb. Symp. quant. Biol.*, **37**, 511-522 (1972).
- ² Yaffe, D., and Dym, H., *Cold Spring Harb. Symp. quant. Biol.*, **37**, 543-547 (1972).
- ³ Holtzer, H., Sanger, J. W., Ishikawa, H., and Strahs, K., *Cold Spring Harb. Symp. quant. Biol.*, **37**, 549-566 (1972).
- ⁴ Brenner, S., *Br. Med. Bull.*, **29**, 269-271 (1973).
- ⁵ Brenner, S., *Genetics*, **77**, 71-94 (1974).
- ⁶ Suzuki, D. T., *Science*, **170**, 695-705 (1970).
- ⁷ Laemmli, U. K., and Favre, M., *J. molec. Biol.*, **80**, 575-599 (1973).
- ⁸ Hirumi, H., Ruski, D. J., and Jones, N. O., *J. ultrastruct. Res.*, **34**, 517-543 (1971).
- ⁹ Epstein, H. F., Waterston, R. H., and Brenner, S., *J. molec. Biol.* (in the press).
- ¹⁰ Rosenbluth, J., *J. Cell Biol.*, **25**, 495-515 (1965).

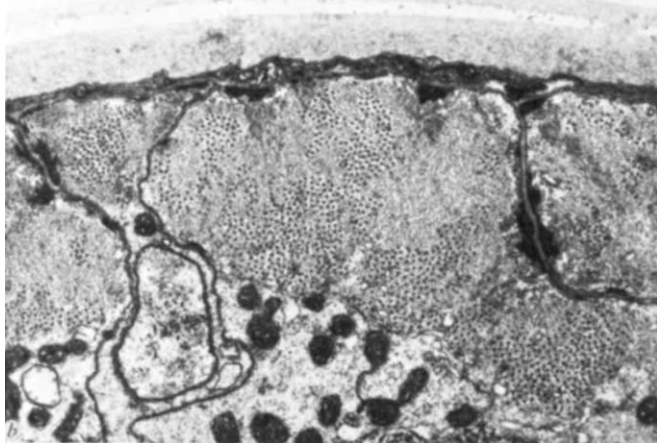
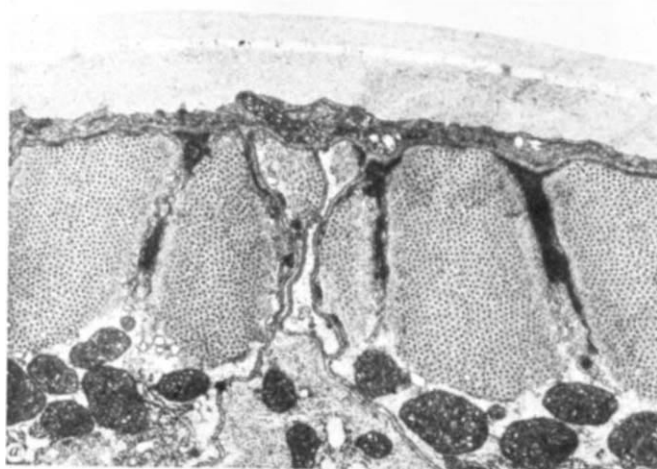


Fig. 2 Nematodes were grown on *E. coli*, OP50, lawns at either temperature. Living adults were cut in half, fixed in cold phosphate-buffered glutaraldehyde, and post-fixed in cold Veronal-buffered osmium tetroxide^{8,9}. Thin sections of body wall muscle cells were photographed on an AEI EM63 electron microscope. The uncalibrated magnification was $\times 14,400$. The dimensions of thick and thin myofilaments are about 2.4 and 0.6 nm, respectively^{9,10}. *a*, E286 at 15° C; *b*, E286 at 25° C.

Genetic complementation after fusion of Tay-Sachs and Sandhoff cells

THE N-acetyl- β -D-glucosaminidase (hexosaminidase) activity in cultured human fibroblasts consists of at least three components (A, B and C)¹⁻³. At least two of these (designated Hex A and Hex B) seem to be closely related, for (1) antiserum against Hex A reacts against Hex B, and *vice versa*^{4,5} (2) in Tay-Sachs disease only Hex A activity is deficient, accompanied by an increase in Hex B activity in certain tissues^{1,6}, and (3) both Hex A and Hex B activities are missing in Sandhoff disease, another autosomal recessive disorder⁷. But in spite of several theories⁸⁻¹¹, the precise relationship between these components remains unknown, as does the nature of the genetic and biochemical relationships between the two diseases. To investigate these questions we have fused Tay-Sachs with Sandhoff fibroblasts and obtained cultures containing heterokaryons which produce a hexosaminidase which is absent from the parent lines. It has the electrophoretic and heat lability characteristics of the Hex A found in normal fibroblasts.

Skin fibroblasts from a patient with Tay-Sachs disease (G.M. 221) and a patient with Sandhoff disease (G.M. 203) were obtained from the Genetic Mutant Repository, Camden, New Jersey, and experiments were carried out with cells at the eighth to eleventh passage. Electrophoretic analysis of extracts of these cells confirmed that the Tay-Sachs fibroblasts lacked Hex A, while the Sandhoff fibroblasts under our experimental conditions has neither Hex A nor Hex B. On rare occasions faint traces of Hex A and

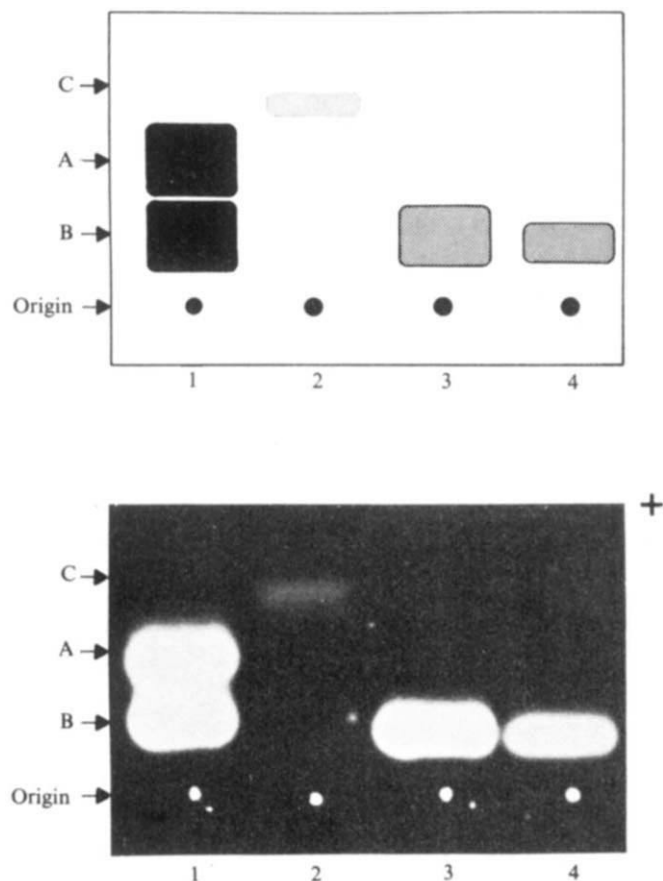


Fig. 1 N-acetyl- β -D-glucosaminidase electrophoresis. (1) Normal control fibroblasts; (2) virus-treated Sandhoff fibroblasts; (3) virus-treated Tay-Sachs cells; and (4) mixture of Sandhoff and Tay-Sachs fibroblasts cultivated together without virus fusion. The electrophoretic analysis was carried out on cell extracts using a slight modification¹³ of the Cellogel method of Rattazzi and Davidson¹⁴.

B could be detected in the Sandhoff fibroblasts when they had been maintained without subculturing for more than 15 d. In addition, both cell lines had, at times, Hex C^{2,3,11}, which in our experience appears only in cultures maintained for 5 or more days without transfer, the actual time apparently depending on the initial cell concentration.

In a series of fusion experiments (Table 1), fibroblasts from the two parental lines, after trypsinisation, were resuspended in Hanks salt solution without glucose. Cells were mixed in a ratio of 2:1, that is 4.8×10^6 Sandhoff cells to 2.4×10^6 Tay-Sachs cells. These cell mixtures, as well as separate aliquots of Tay-Sachs and Sandhoff fibroblasts, were exposed to β -propiolactone-inactivated Sendai virus¹² in the cold for 15 min. Cells were then resuspended in medium (Eagle's minimal essential medium with non-essential amino acids, penicillin, streptomycin and 15% foetal calf serum) and plated in a series of 60 mm plastic Petri dishes (10^4 – 10^6 cells per dish). In addition, mixtures of the parental lines (2 Sandhoff:1 Tay Sachs) not exposed to virus were plated into dishes as another control.

Sixteen to 24 h after fusion, cover slips in dishes containing the virus-treated Sandhoff–Tay-Sachs cell mixtures were fixed and analysed for the presence of multinucleated cells (Table 1). Cells from experimental and control dishes were collected at various times after fusion for the electrophoretic analysis of hexosaminidase activity (Table 1). The cells were washed twice with phosphate-buffered saline (PBS), trypsinised, and, after centrifugation, washed again with PBS. Each pellet was suspended in 0.05–0.2 ml (de-

pending on cell concentration) of 0.01 M citrate-phosphate buffer, pH 7.0, and disrupted by ultrasonication (three 10-s treatments). The ruptured cells were centrifuged for 10 min at $5,000g$ at 3 – 5°C and the supernatant was used for biochemical studies.

Without virus treatment, the cocultivation of the parental lines in a ratio of 2:1 (Sandhoff to Tay-Sachs) for as long as 16 d yielded Hex B, and in time Hex C, but no Hex A (Fig. 1). Virus-treated Tay-Sachs fibroblasts also showed Hex B, and on prolonged culture the C band, but never Hex A. The virus-treated Sandhoff cells showed neither Hex A nor Hex B, but on prolonged culture they also had Hex C.

In contrast, the virus-treated mixtures of parental lines had, in addition to the expected Hex B, a band comigrating with Hex A (Fig. 2). The latter appeared in one experiment as early as 24 h and in all cases seemed to increase to a maximum between 3 and 6 d after fusion (Table 1 and Fig. 2). The activity of Hex A seemed to correlate with the number of multinucleated cells present in the culture and declined when the nondividing heterokaryons had been overgrown by mononuclear parental cells.

As this new A-like band might represent a modified form of Hex B or C, its thermal lability was examined. Cell extracts were incubated under conditions in which the Hex A activity is destroyed while most of the Hex B activity remains. We found that the novel component formed by the virus-treated Sandhoff–Tay-Sachs mixture had the heat lability of the Hex A found in normal fibroblasts (Fig. 3).

We suggest that the novel band produced by virus-induced heterokaryons is the Hex A enzyme lacking in both parental lines. A more positive identification awaits the development of sensitive and specific assays involving antibodies or labelled substrates.

It is conceivable that the substance responsible for the appearance of this novel band is passed through the medium from heterokaryon to deficient parental cells. But, even when Sandhoff or Tay-Sachs cells were exposed for 2–11 d to medium in which virus-treated cell mixtures had been proliferating for at least 3 d, no evidence of Hex A was found. Furthermore, neither incubation of mixtures of cell extracts from the two parental lines at 37°C for up to 144 h, nor prolonged exposure of lysates from one parent line to proliferating cells of the other, resulted in the production of Hex A.

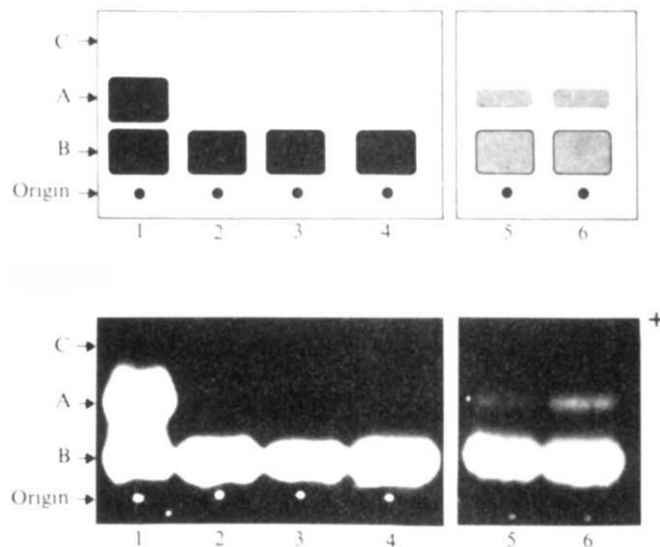


Fig. 2 N-acetyl- β -D-glucosaminidase electrophoresis. (1) Extract from normal fibroblasts and (2–6) extracts from virus-treated mixtures of Tay-Sachs and Sandhoff fibroblasts 16, 24, 48, 72, and 144 h after fusion. The novel band shown in this figure comigrates with Hex A when mixed with extracts of control fibroblasts.

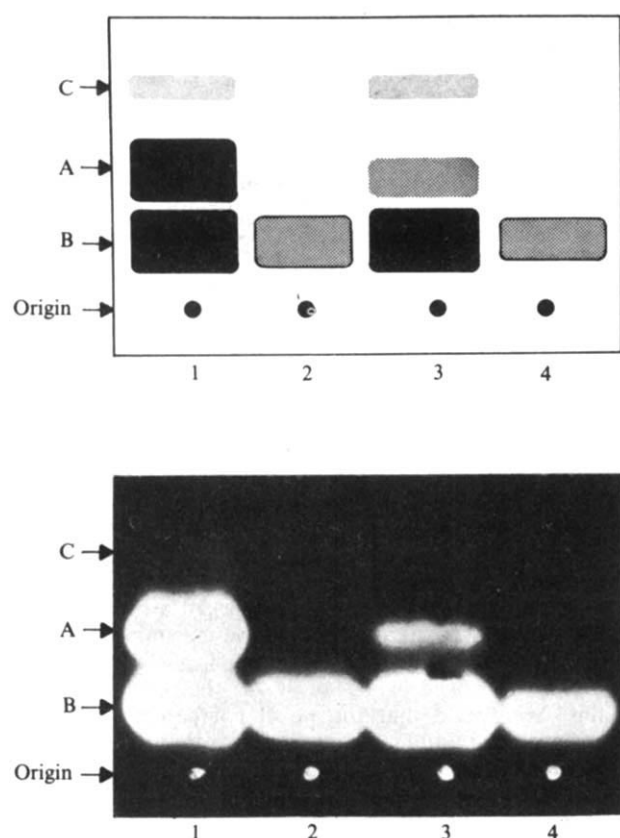


Fig. 3 Cellogel electrophoresis of cell extracts before and after incubation in 0.01 M phosphate-citrate buffer; pH 7.0, for 45 min at 52° C. Extract of normal fibroblasts before (1) and after (2) incubation; extracts of virus-treated Tay-Sachs-Sandhoff mixture before (3) and after (4) incubation.

Our results (Table 1) suggest that this enzyme persists in these heterokaryons for as long as 16 d, at a time when the cell population contains relatively few heterokaryons. Conceivably the product responsible for the appearance of the activity passes from heterokaryon by cell-mediated contact to surrounding parental cells or alternatively is stored for prolonged periods, as reported for β -glucuronidase¹⁵.

The mechanism underlying the appearance of the novel enzyme, believed to be Hex A, is uncertain. Perhaps the Sandhoff cells provide either an enzyme to convert the Hex B of Tay-Sachs origin into Hex A, or a subunit essential to the formation of Hex A. On the other hand, the Tay-Sachs cells might stabilise in some way a labile Hex B and Hex A of Sandhoff origin.

We have not attempted to isolate mononuclear hybrid cells because of the lack of an effective means to select against either parental cell.

Nevertheless, our studies of the heterokaryons derived

from Tay-Sachs and Sandhoff cells show that even in the absence of selection this approach can reveal intergenic complementation. Complementation tests have already provided evidence of genetical heterogeneity contributing to the phenotypes of galactosemia, xeroderma pigmentosum, and maple syrup urine disease¹⁶⁻¹⁸. The study of the interaction between different genomes within the heterokaryon, not only reveals genetical heterogeneity, but also indicates directions to follow in pursuit of the underlying disease mechanism.

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- ¹ Okada, S., Veath, M. L., Leroy, J., and O'Brien, J. S., *Am. J. Human Genet.*, **23**, 55 (1971).
- ² Gilbert, F., Kucherlapati, R., and Ruddle, F. H., *Abstract, The American Society of Human Genetics*, October 24-27, 1973, 29A.
- ³ Poenaru, L., and Dreyfus, J. C., *Clin. chim. Acta*, **43**, 439 (1973).
- ⁴ Carroll, M., and Robinson, D., *Biochem. J.*, **126**, 17 (1972).
- ⁵ Srivastava, S. K., and Beutler, E., *Biochem. biophys. Res. Commun.*, **47**, 753 (1972).
- ⁶ Okada, S., and O'Brien, J. S., *Science*, **165**, 698 (1969).
- ⁷ Sandhoff, K., Andreae, U., and Jatzkewitz, H., *Life Sci.*, **7**, 283 (1968).
- ⁸ Goldstone, A., Konecny, P., and Koenig, H., *FEBS Lett.*, **13**, 68 (1971).
- ⁹ Snyder, jun., P. D., Krivit, W., and Sweeley, C. C., *J. Lipid Res.*, **13**, 128 (1972).
- ¹⁰ Robinson, D., and Carroll, M., *Lancet*, **i**, 322 (1972).
- ¹¹ Ropers, H. H., and Schwantes, U., *Humangenetik*, **20**, 167 (1973).
- ¹² Neff, J. N., and Enders, J. F., *Proc. Soc. exp. Biol. Med.*, **127**, 260 (1968).
- ¹³ Thomas, G. H., Taylor, H. A., Reynolds, L. W., and Miller, C. S., *Pediat. Res.*, **7**, 751 (1973).
- ¹⁴ Rattazzi, M. C., and Davidson, R. G., in *Antenatal diagnosis*, (edit. by Dorfman, A.), 207 (The University of Chicago Press, Chicago, 1972).
- ¹⁵ Langunoff, D., Nicol, D. M., and Pritzl, P., *Lab. Invest.*, **29**, 449 (1973).
- ¹⁶ Nadler, H. L., Chacko, C. M., and Rachmeler, M., *Proc. natn. Acad. Sci. U.S.A.*, **67**, 976 (1970).
- ¹⁷ De Weerd-Kastelein, E. A., Keijzer, W., and Bootsma, D., *Nature new Biol.*, **238**, 80 (1972).
- ¹⁸ Lyons, L. B., Cox, R. P., and Dancis, J., *Nature*, **243**, 533 (1973).

Solvent exposure of specific nuclei of angiotensin II determined by NMR solvent saturation method

CONFORMATIONAL analysis of peptides in solution by nuclear magnetic resonance (NMR) spectroscopy generally involves determination of the relative exposure to solvent of specific NH hydrogens¹⁻⁴. The four methods used so far are based on: (1) rates of NH proton exchange with labile hydrogens of the solvent⁵⁻⁶; (2) temperature dependence of chemical shifts of NH resonances⁷⁻⁸; (3) dependence of NH chemical shifts on the composition of a suitable solvent mixture⁹⁻¹¹, and (4) degree of resonance broadening when a paramagnetic substance is added¹². Each method has limitations. Proton exchange rates reflect not only exposure to solvent,

Table 1 Activity of novel-N-acetyl- β -D-glucosaminidase in cultures containing virus-induced heterokaryons of Tay-Sachs and Sandhoff fibroblasts

Experiment	% Multi-nucleated cells*	Days after fusion									
		1	2	3	4	5	6	7	8	-	16
1	20	±	+	+			+	+			
2	9	-	-	+			+	+	+		+
3	10		+	+	+	+	+	-	-		

* 16-24 h after fusion.

but also proximity to functional groups of the peptide which catalyse exchange. Factors which determine the temperature dependence of NH chemical shifts are as yet poorly understood. Changes in solvent composition can alter the conformation of the peptide¹¹. Paramagnetic ions may associate preferentially with the solvent or with specific sites on the peptide.

We suggest here a method which offers the advantage of measuring the solvation of both NH and CH hydrogens while not physically altering molecular structure. This involves monitoring intensity changes of solute resonances resulting from saturation of the solvent resonance. Intensities of solute resonances originating from exposed labile hydrogens exchanging rapidly with the solvent are diminished by transfer of saturation¹³⁻¹⁶, whereas resonances of exposed nonexchangeable hydrogens are enhanced by a positive nuclear Overhauser effect (NOE)¹⁷. Although the method is generally applicable to any solute-solvent pair having suitable nuclei, it is particularly appropriate to investigations of peptides and other biomolecules in aqueous solution. Here we describe its application to the interaction of water with the pressor hormone (Asn¹, Val³) angiotensin II (AII'), whose primary structure appears in Fig. 1. Materials and the correlation spectroscopy technique used have been described before^{16,18,19}.

Transfer of saturation is governed by equation (1)

$$(M_{\alpha}^{\alpha} - M_{\alpha}^{\beta})/M_{\alpha}^{\alpha} = [T_{1\alpha}/(T_{1\alpha} + \tau_{\alpha})] [(M_{\beta}^{\beta} - M_{\beta}^{\alpha})/M_{\beta}^{\beta}] \quad (1)$$

which was derived from modified Bloch equations using a procedure analogous to that used by Gupta and Redfield²⁰. The α and β states refer to solute and solvent nuclei, respectively, which are chemically exchanging. $T_{1\alpha}$, τ_{α} , M_{α}^{α} and M_{α}^{β} are the spin-lattice relaxation time, life time, observed magnetisation and equilibrium magnetisation of the α nucleus, respectively. Analogous notation is used for the β nucleus. $(M_{\alpha}^{\beta} - M_{\alpha}^{\alpha})/M_{\alpha}^{\alpha}$ is the fractional decrease in resonance intensity of the α resonance resulting from double irradiation of the β resonance, whose intensity is diminished by a factor of $(M_{\beta}^{\beta} - M_{\beta}^{\alpha})/M_{\beta}^{\beta}$. Complete saturation of the β state causes a fractional decrease of the α resonance equal to $T_{1\alpha}/(T_{1\alpha} + \tau_{\alpha})$, which is significant only if the pseudo-first order rate constant for exchange of the α nucleus, $1/\tau_{\alpha}$, is comparable with or greater than its relaxation rate, $1/T_{1\alpha}$. Measurement of the extent of saturation transfer and $T_{1\alpha}$ facilitates estimation of the exchange rate.

Saturation of the solvent resonance also results in positive NOEs for resonances originating from solute hydrogens exposed to the solvent or in close proximity to solute hydrogens experiencing saturation. Saturation of solute resonances can result either from exchange-mediated transfer of saturation from the solvent or from coincidence of solute and solvent resonances (which is usually also caused by rapid exchange with the solvent). Three mechanisms can contribute to the observed NOE: (1) direct dipole-dipole interaction between solute and solvent hydrogens (such intermolecular dipole-dipole effects have been observed, and a theoretical treatment of this phenomenon has been derived by Krishna and Gordon)²¹, (2) dipole-dipole interaction between the monitored hydrogen and a nearby partially or completely saturated solute hydrogen, and (3) scalar coupling between the monitored hydrogen and a solute hydrogen which is exchanging with the solvent at a rate comparable to the chemical shift difference between the two coupled solute hydrogens¹⁷. This exchange-modulated scalar coupling would yield a negative NOE (decrease in resonance intensity), whereas the dipolar mechanisms yield positive NOEs except when long correlation times are encountered (for example, macromolecules)²².

Figure 1a shows the region of the spectrum of AII' to low field of the water resonance when saturation power is applied 1,200 Hz to high field of the solvent peak. Resonance assignments are those obtained in this

laboratory^{16,23,24} and by Bleich, *et al.*^{25,26}. Saturation of the water resonance yields the spectrum shown in Fig. 1b. Figure 1c shows the difference (amplified) between the solvent saturated and the off resonance irradiated spectra (Fig. 1b-Fig. 1a). Negative peaks originate from solvent exposed labile hydrogens experiencing transfer of saturation—Arg² peptide NH , Asn¹ *trans*-amide NH , and Arg² guanidino NH s. The Arg² peptide NH , Asn¹ amide NH and Arg² guanidino NH resonances decreased in intensity by factors of 0.50 ± 0.05 , 0.09 ± 0.03 and 0.07 ± 0.02 , respectively. Positive peaks originate from solute hydrogens experiencing a positive NOE—His⁶ C_2H , His⁶ C_1H , Tyr⁴ *o*-CH and perhaps also the Phe⁸ C_6H_5 (overlap with the His⁶ C_1H peak obscures this resonance). The His⁶ C_2H and Tyr⁴ *o*-CH peaks experienced fractional enhancements of resonance intensities of 0.17 ± 0.05 and 0.12 ± 0.03 , respectively. Larger NOEs might be obtained by minimising relaxation pathways not associated with solute-solvent interactions, for example, by degassing the sample or using partially deuterated samples. Perturbation of His⁶ and Tyr⁴ peptide NH peaks results from partial decoupling of their corresponding $\alpha\text{-CH}$ resonances, which are close to the solvent peak.

Transfer of saturation indicated that the NH protons of Arg² exchange rapidly with the solvent and are therefore

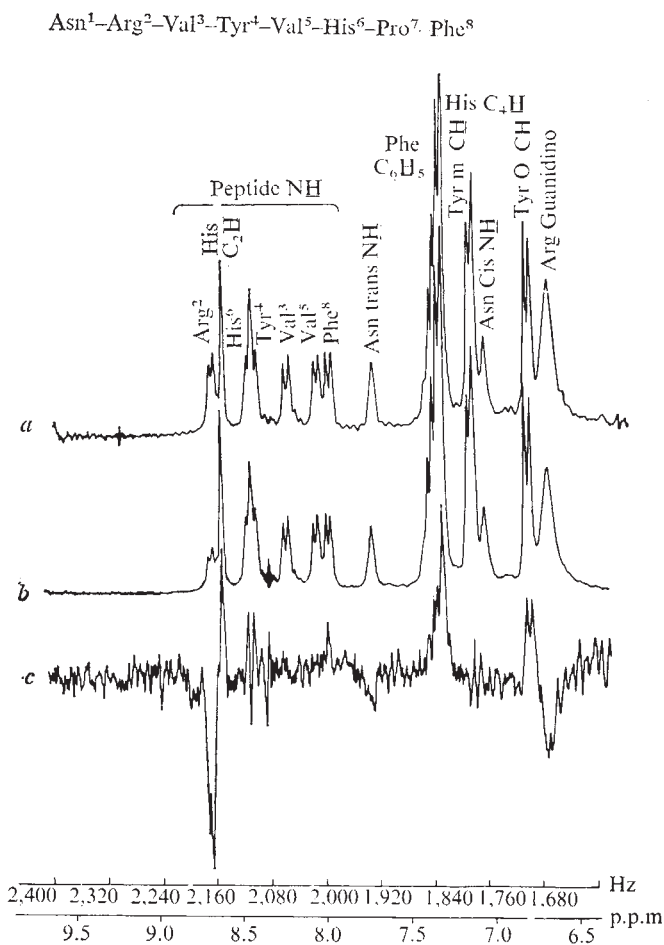


Fig. 1 a, Low field region of the 250 MHz PMR spectrum of AII' (6.9% (w/v)) in H_2O , pH 3.0 ± 0.1 , at 31.5°C . The spectrum was obtained by correlation spectroscopy (twenty scans) with saturating rf power applied 1,200 Hz to high field of the solvent resonance. Chemical shifts are referred to the methyl resonance of sodium 2,2-dimethyl-2-silapentane 5-sulphonate. b, Same as (a) but with the solvent peak saturated. c, Spectrum (b) minus spectrum (a) amplified thirteenfold.

probably well solvated. Rapid exchange of these hydrogens has been noted previously^{16,23,26}. Partial saturation of the Asn¹ *trans*-amide NH suggests that this hydrogen is also exposed to the solvent. Taken together the data for Asn¹ and Arg² suggest that the two N-terminal residues of AII are relatively well solvated. This conclusion is consistent with our report that the *pK_a* of the Asn¹ amino group is comparable with that of similar solvated functional groups^{23,24}.

Observations of positive NOEs indicates that the dipolar mechanisms dominate over any exchange-modulated scalar coupling which might be contributing to the relaxation of the His² imidazole CH and Tyr⁴ *o*-CH protons. These protons must be in intimate contact with the solvent and (or) the nearby exchangeable protons on the side chains of these residues must be substantially saturated as a result of rapid exchange with the solvent. In either case a solvated environment is indicated for these CH protons since dipolar relaxation depends on the inverse sixth power of the internuclear distance. Solvation of the imidazole and phenol of AII' is consistent with the normal *pK_a*s of these groups^{23,24,27,28}. Failure to observe an NOE from the Tyr⁴ *m*-CH hydrogens may indicate that this portion of the phenol group is not in contact with water. These studies show how solvent saturation effects can delineate the extent of solvation of both labile and nonexchanging hydrogens.

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- ¹ Urry, D. W., and Ohnishi, M., in *Spectroscopic approaches to biomolecular conformation* (edit. by Urry, D. W.), cha. 7 (American Medical Association Press, Chicago, Illinois, 1970).
- ² Bovey, F. A., Brewster, A. I., Patel, D. J., Tonelli, A. E. and Torchia, D. A., *Accounts of Chem. Res.*, **5**(6), 193-200 (1972).
- ³ Kopple, K. D., and Schamper, T. J., in *Chemistry and biology of peptides* (edit. by Meienhofer, J.), 75-80 (Ann Arbor Publishers, Ann Arbor, 1972).
- ⁴ Stern, A., Gibbons, W. A., and Craig, L. C., *Proc. natn. Acad. Sci. U.S.A.*, **61**, 734-741 (1968).
- ⁵ Hvidt, A., and Nielson, S. O., *Adv. Protein Chemistry*, **21**, 287-386 (1966).
- ⁶ Molday, R. S., Englander, S. W., and Kallen, R. G., *Biochemistry*, **11**, 150-158 (1972).
- ⁷ Kopple, K. D., Ohnishi, M., and Go, A., *J. Am. chem. Soc.*, **91**, 4264-4272 (1969).
- ⁸ Ohnishi, M., and Urry, D. W., *Biochem. biophys. Res. Commun.*, **36**, 194-202 (1969).
- ⁹ Pitner, T. P., and Urry, D. W., *J. Am. chem. Soc.*, **94**, 1399-1400 (1972).
- ¹⁰ Pitner, T. P., and Urry, D. W., *Biochemistry*, **11**, 4132-4137 (1972).
- ¹¹ Walter, R., and Glickson, J. D., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 1199-1203 (1973).
- ¹² Kopple, K. D. and Schamper, T. J., *J. Am. chem. Soc.*, **94**, 3644-3646 (1972).
- ¹³ Forsen, S., and Hoffman, R. A., *J. chem. Phys.*, **39**, 2892-2901 (1963).

- ¹⁴ Hoffman, R. A., and Forsen, S., in *Progress in nuclear magnetic resonance spectroscopy* (edit. by Emsley, J. W., Feeney, J., and Sutcliffe, L. H.), Volume 1, 15-204 (Pergamon Press Ltd, Oxford, 1966).
- ¹⁵ Von Dreele, P. H., Brewster, A. I., Dadok, J., Scheraga, H. A., Bovey, F. A., Ferger, M. F., and Du Vignaud, V., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 2169-2173 (1972).
- ¹⁶ Glickson, J. D., Dadok, J., and Marshall, G. R., *Biochemistry*, **13**, 11-14 (1974).
- ¹⁷ Noggle, J. H., and Schirmer, R. E., *The nuclear overhauser effect*, (Academic Press, New York, 1971).
- ¹⁸ Dadok, J., and Sprecher, R. F., *J. Magn. Res.*, **13**, 243-248 (1974).
- ¹⁹ Gupta, R. K., Ferretti, J. A., and Becker, E. D., *J. Magn. Res.*, **13**, 275-290 (1974).
- ²⁰ Gupta, R. K., and Redfield, A. G., *Biochem. biophys. Res. Commun.*, **41**, 273-281 (1970).
- ²¹ Krishna, N. R., and Gordon, S. L., *J. chem. Phys.*, **58**, 5687-5696 (1973).
- ²² Balaram, P., Bothner-By, A. A., and Dadok, J., *J. Am. chem. Soc.*, **94**, 4015-4017 (1972); Balaram, P., Bothner-By, A. A., and Breslow, E., *ibid.*, 4017-4018 (1972).
- ²³ Glickson, J. D., Cunningham, W. D., and Marshall, G. R., in *Chemistry and biology of peptides* (edit. by Meienhofer, J.), 563-570 (Ann Arbor Publishers, Ann Arbor, 1972).
- ²⁴ Glickson, J. D., Cunningham, W. D., and Marshall, G. R., *Biochemistry*, **12**, 3684-3692 (1973).
- ²⁵ Bleich, H. E., Galardy, R. E., and Printz, M. P., *J. Am. chem. Soc.*, **95**, 2041-2042 (1973).
- ²⁶ Bleich, H. E., Galardy, R. E., Printz, M. P., and Craig, L. C., *Biochemistry*, **12**, 4950-4957 (1973).
- ²⁷ Paiva, T. B., Paiva, A. C. M., and Scheraga, H. A., *Biochemistry*, **2**, 1327-1334 (1963).
- ²⁸ Zimmer, S., Haar, W., Maurer, W., Ruterjans, H., Femandjian, S., and Fromageot, P., *Eur. J. Biochem.*, **29**, 80-87 (1972).

Ferritin synthesis in normal and leukaemic leukocytes

SERUM ferritin concentration is normally directly related to body iron stores^{1,2} but abnormally high values are also found in patients with leukaemia³. The greatest amounts are present in patients with acute myeloblastic leukaemia and in this condition the concentration within circulating leukocytes is about six times higher than in normal leukocytes⁴. We have demonstrated an abnormally high rate of overall ferritin synthesis in leukaemic cells. Previous studies in other tissues have shown that ferritin synthesis is closely related to iron supply, but this does not seem to be the case in either normal or leukaemic leukocytes where production of the protein is independent of iron concentration and the protein formed contains little, if any, iron.

Peripheral blood leukocytes were obtained from healthy adults and patients with acute myeloblastic leukaemia by sedimentation and differential centrifugation⁴. In synthesis experiments the white cell pellet was suspended in Eagle's minimum essential medium (MEM) at a concentration of 2×10^6 cells ml⁻¹, to which 20% foetal calf serum and 1 μ Ci ¹⁴C-leucine were added. Cultures were incubated in triplicate at 37° C under 5% CO₂ in air for 22 h. Controls consisted of omitting the cells from the incubation medium or the addition of cycloheximide at a concentration of 10⁻⁴ M. At the end of the incubation, the cells were sonicated and dialysed against five changes of 0.3% saline for 3 h. One hundred micrograms of human spleen ferritin were added and the total volume brought to 9 ml. A 2 ml aliquot was removed and the protein precipitated with 10% trichloroacetic acid followed by heating at 90° C for 15 min. The precipitate was washed with trichloroacetic acid and finally dissolved in 0.1 M NaOH before scintillation counting. Ferritin was isolated by heating the cell extract containing carrier ferritin at 70° C for 10 min, followed by centrifugation at 1,300g for 10 min. Anti-human spleen ferritin was then added to the supernatant, incubated at 37° C for 1 h and left at 4° C overnight. The precipitate was

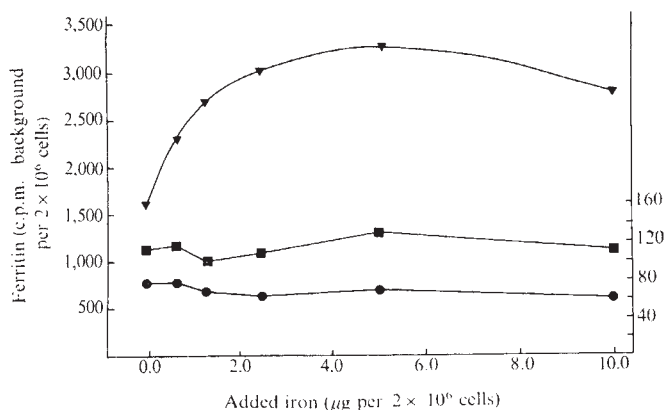


Fig. 1 ^{14}C -leucine incorporation into ferritin at different iron concentrations. The iron content of the basal medium is $0.67 \mu\text{g}$ per 2×10^6 cells. ▼, Chang cells; ●, leukaemic leukocytes (left hand scale). ■, Normal leukocytes (right hand scale).

washed three times with 0.9% saline and dissolved in 0.5 M HCl before scintillation counting. Chang liver cells⁵ (Flow Laboratories) were grown in MEM with 10% foetal bovine serum. Ferritin synthesis was measured under the same conditions and with the same cell concentration used with leukocytes. Sucrose density gradient fractionation of ferritin was carried out by the method of Drysdale and Munroe⁶. Twenty fractions of 0.5 ml were collected and ferritin was determined either by immunoradiometric assay or by the absorbance at 280 nm.

^{14}C -leucine incorporation into ferritin and total protein is expressed as c.p.m. per 10^6 cells. In seven normal subjects, the mean ferritin uptake was 54.2 ± 5.5 c.p.m. and the mean protein uptake $1,876 \pm 190$ c.p.m. The protein-ferritin ratio was 35.7 ± 4.1 . In seven patients with acute myeloblastic leukaemia, the mean ferritin uptake was 230.8 ± 36.6 c.p.m. and protein uptake $7,526 \pm 1,140$ c.p.m. with a protein-ferritin ratio of 33.0 ± 2.3 . Increasing the iron concentration in the medium from 0 to $10.0 \mu\text{g ml}^{-1}$ had no effect on ferritin synthesis in either normal or leukaemic cells. In experiments using Chang liver cells, a similar increase in iron concentration in the medium resulted in stimulation of ferritin synthesis (Fig. 1).

The largest amounts of purified human spleen ferritin appeared between fractions 8 and 11, in nine separate experiments. In three experiments using normal leukocyte extract, and six experiments using leukocyte extracts from patients with acute myeloblastic leukaemia, the maximum concentration of ferritin appeared in fraction 2 to 4 (Fig. 2) corresponding to apoferritin.

These results demonstrate increased ferritin synthesis by leukaemic cells and supports the suggestion that this is the cause of the raised serum and leukocyte ferritin concentration⁴ in leukaemia patients. Experimental studies on the control of ferritin synthesis have been carried out largely on liver cells and in this tissue, ferritin synthesis is closely related to iron supply⁶. Ferritin synthesis *in vitro* by HeLa cells is also stimulated by iron⁷ and *in vivo* studies have shown a similar iron dependence of ferritin synthesis by rat hepatoma⁸. Ferritin synthesis in Chang cells shows marked stimulation by iron, but without any increase in overall protein synthesis. In normal leukocytes, ferritin synthesis does not seem to be stimulated by increasing concentrations of iron, and leukaemic cells show the same insensitivity. In leukaemic cells, ^{14}C -leucine incorporation into ferritin takes place at about four times the normal rate and this seems to be a reflection of increased protein synthesis by these cells.

The characteristics of leukocyte ferritin on density gradient centrifugation suggests that this is either apoferritin or protein with a very low iron content. It has

been known for some time that the ferritins in different tissues may vary in their physical characteristics and probably their subunit composition. Linder-Horowitz *et al.*⁹ showed that the stimulation of ferritin synthesis by iron varies in different organs, being more marked in liver and heart than in kidney. While the iron content of liver ferritin remains constant that of heart and kidney ferritin falls with increased synthesis of the apoprotein. In the female rat heart, where there are two separate ferritins, the electrophoretically slow variant shows less response than the fast variant to stimulation by iron. In a study of abnormal ferritins in rat hepatomas, Linder *et al.*⁸ showed that protein induction by iron was less than in normal regenerating liver. Increasing growth rate of the tumours was associated with decreased iron uptake, a decreased concentration of ferritin in the tumour and a lower iron content of the ferritin. It was suggested that ferritin of low iron content might be characteristic of tissues undergoing rapid cell division but the possibility also exists that in malignant tissue, expression of an alternative gene locus for ferritin

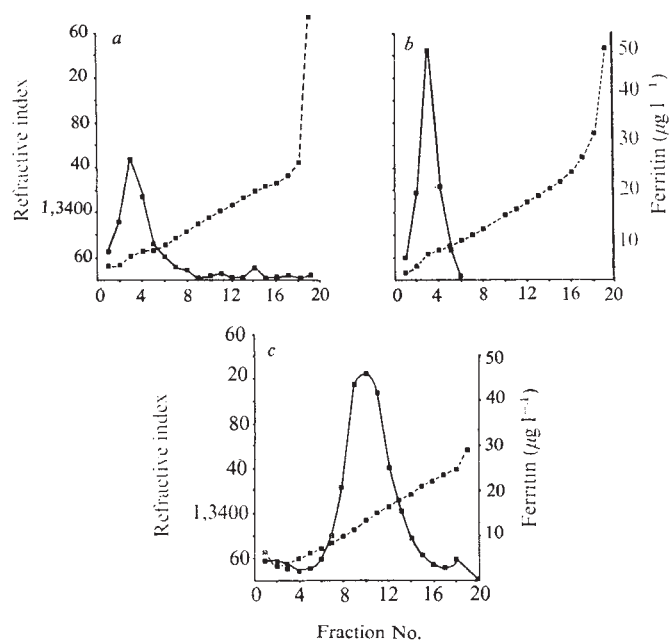


Fig. 2 Density gradient sedimentation of ferritin. ■—■ ferritin; ■---■, refractive index of medium. a, Normal leukocytes; b, leukaemic leukocytes; c, human spleen ferritin.

occurs and that this is associated with a low affinity of the protein for iron. The observation that synthesis in both normal and leukaemic leukocytes is not stimulated by iron and that the protein has the characteristics of apoferritin suggests the existence of a specific leukocyte ferritin. Its increased synthesis in acute myeloblastic leukaemia and its subsequent release into the plasma reflects the overall increase in protein synthesis by leukaemic cells and may provide a useful marker for the progress of the disease.

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¹ Jacobs, A., Miller, F., Worwood, M., Beamish, M. R., and Wardon, C. A., *Br. Med. J.*, **4**, 206-208 (1972).

² Walters, G. O., Miller, F., and Worwood, M., *J. clin. Path.*, **26**, 770-772 (1973).

- ³ Jones, P. A. E., Miller, F., Worwood, M., and Jacobs, A., *Br. J. Cancer*, **27**, 212-217 (1973).
⁴ Worwood, M., Summers, M., Miller, F., Jacobs, A., and Whittaker, J. A., *Br. J. Haemat.* (in the press).
⁵ Chang, R. S., *Proc. Soc. exp. Biol. Med.*, **87**, 440-443 (1954).
⁶ Drysdale, J. W., and Munro, H. N., *J. biol. Chem.*, **241**, 3630-3637 (1966).
⁷ Chu, L. L. H., and Fineberg, R. A., *J. biol. Chem.*, **244**, 3847-3854 (1969).
⁸ Linder, M., Munro, H. N., and Morris, H. P., *Cancer Res.*, **30**, 2231-2239 (1970).
⁹ Linder-Horowitz, M., Ruettinger, R. T., and Munro, H. N., *Biochim. biophys. Acta*, **200**, 442-448 (1970).

β , γ Unsaturated amino acids as irreversible enzyme inhibitors

IRREVERSIBLE enzyme inhibitors whose mechanisms of action are based on the k_{cat} term rather than on K_s are highly specific. Inhibitors of this type possess hidden reactive moieties which are unmasked enzymatically. On generation, the reactive product engages in a chemical reaction with an active site residue resulting in irreversible inactivation of the enzyme. Thus, the enzyme produces its own irreversible

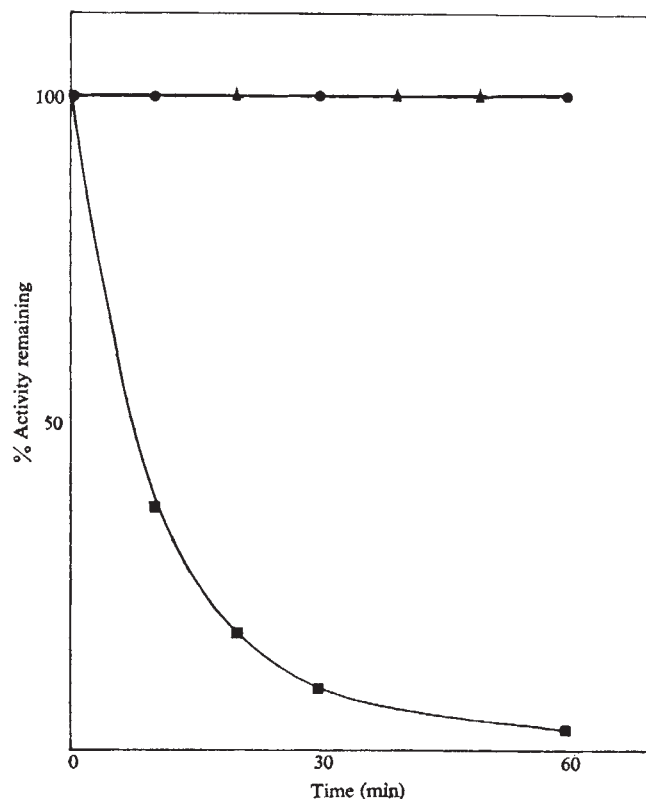


Fig. 1 Inactivation of aspartate amino transferase by AMB. Aspartate amino transferase from pig heart (Sigma Chemical Co.), specific activity 234 units mg^{-1} (where one unit of enzyme will convert 1 μmol of α -ketoglutarate to glutamic acid per min at 25°C) was used. In these experiments 0.008 units of enzyme ($\blacksquare$) were incubated at 25°C in 0.1 M potassium phosphate buffer, pH=7.6 with 10 mM AMB. At the times indicated, 5 μl of the enzyme was removed and its activity determined by the standard assay system containing aspartic acid, α -ketoglutaric acid, NADH and malic dehydrogenase⁶. Identical experiments were performed in the presence of 0.2 M aspartic acid ($\blacktriangle$) and in the absence of AMB ($\bullet$) (control). The activity of the inactivated enzyme was not restored by dialysis against the phosphate buffer with several changes over a 36 h period attesting to the enzymes being irreversibly inactivated. Furthermore, the activity of the inactivated enzyme could also not be restored by incubation with 1 mM pyridoxal phosphate for several hours.

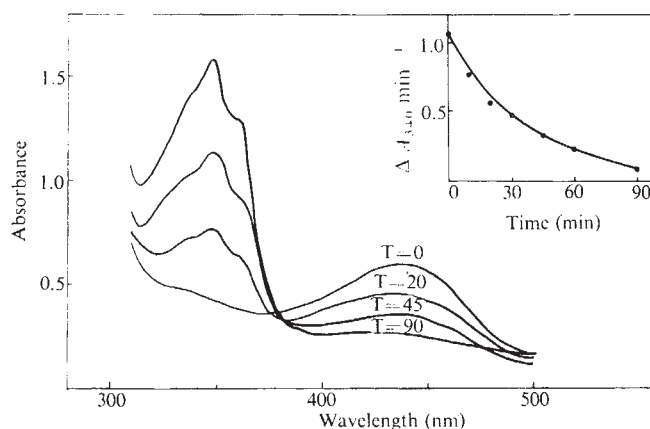


Fig. 2 The ultraviolet spectra and inactivation of aspartate amino transferase by AMB. In a 1 cm cuvette 936 units of enzyme in 1 ml 0.1 M potassium phosphate at pH=6.0 was charged with 10 mM AMB at 25°C. The ultraviolet spectra of the sample were determined at the indicated times on a Cary 118 double beam spectrophotometer. The activity of an aliquot of the enzyme was determined by the standard method⁶ and compared with an untreated control. Inset shows the inactivation profile at the indicated times.

inhibitor from a chemically unreactive substrate. Inhibitors of this type will be referred to as k_{cat} inhibitors, and several synthetic inhibitors of this type have been reported¹. Here I describe an example of a naturally occurring molecule which acts by this mechanism: specifically, the irreversible inhibition of soluble, pyridoxal linked, L-aspartate amino transferase by the bacterial toxin L-2-amino-4-methoxy-trans-3-butenoic acid (AMB) isolated from *Pseudomonas aeruginosa*².

Incubation of soluble L-aspartate amino transferase with AMB led to the inactivation of the enzyme as shown in Fig. 1. The substrate, in this case L-aspartic acid, protects against this inactivation when co-incubated with AMB (Fig. 1) and is consistent with the view that AMB is an active-site titrant. Also, activity of the inactivated enzyme cannot be restored by dialysis. This observation means that the mode of inhibition is irreversible.

The assertion that AMB requires chemical activation suggests that no inhibition should result in the absence of enzymatic conversion. This is confirmed in that neither holoenzyme in the pyridoxamine form nor apoenzyme, are in the least affected by AMB. The notion that the inactivation step requires enzymatic conversion can be directly demonstrated by following the ultraviolet absorption changes of the holoenzyme as a function of its inactivation (Fig. 2). As the inactivation proceeds, both the activity and the spectrum of the enzyme were recorded and as can be seen, the fall of the pyridoxal phosphate imine peak (approximately 440 nm) and the rise of the new peaks at approximately 350 nm are simultaneous with the inactivation course. The appearance of the peaks at 350 nm are unusual and cannot be pyridoxamine phosphate ($\lambda_{max}=325$ nm) (ref. 3). These new peaks represent a reaction product, or complex, between the converted AMB and cofactor. This may mean that the inhibitor functions by reacting with the cofactor. This was tested by trying to resolve the inactivated holoenzyme into apoenzyme and cofactor by the usual method⁴. The 'resolved' enzyme still retained an absorption peak centred at 350 nm, representing about 30% of the total. This peak cannot be diminished even under forced resolution conditions. Of primary importance is that the resolved enzyme cannot be reactivated by incubation with fresh pyridoxal phosphate, therefore the primary action of AMB must involve a chemical reaction with an active-site amino acid residue. Of secondary importance is the formation of a further reaction pro-

duct(s) with the cofactor. Had a chemical reaction simply ensued between the cofactor and AMB, 70% of the activity should have been regained after fresh cofactor was added to the resolved enzyme.

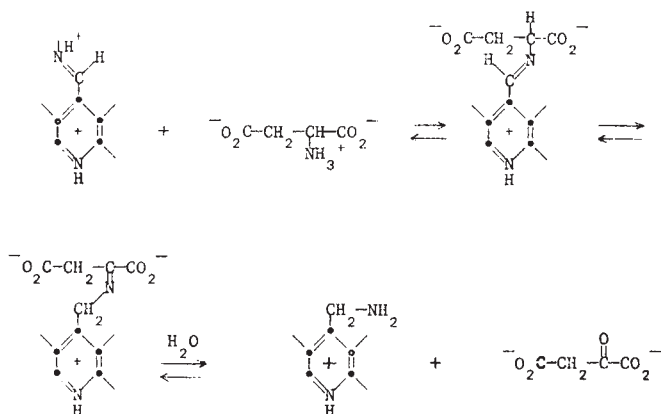


Fig. 3 Initial steps in the catalysis with aspartate and α -ketoglutarate as substrate.

The initial steps in the catalytic process effected by aspartate amino transferase with aspartate and α -ketoglutarate as substrates are shown in Fig. 3 (ref. 5). With AMB as a substrate the conversions shown below can intervene once the α C—H bond is cleaved (Fig. 4). That both (B+B') and C are exceedingly reactive chemically is important because they can act as Lewis acids in the Michael reaction. The carbon atoms indicated with stars * designate where reaction would occur. The conversion shown in equation 1 is preferred on the basis of its being a normally occurring one for the enzyme, and is more consistent with the spectral changes which occur during the course of the inactivation process. Also, it is apparent that these proposed reactive intermediates are chemically bifunctional; for example, B', being a conjugated enol ether, can undergo two successive reactions with nucleophiles. This is precisely what is required here, because a minimum of two reactions must occur—one with an active-site

residue and one with the cofactor—to account for the spectral changes and the inactivation process.

In conclusion, AMB has been shown to be a potent irreversible inhibitor of the k_{cat} type, the first naturally-occurring toxin to be reported as such. It is likely that this is in no sense unusual but that other β,γ unsaturated acids will be found to be irreversible inhibitors of many pyridoxal-linked enzymes involved in amino acid metabolism, and that other naturally occurring toxins will also be found to be k_{cat} inhibitors.

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- ¹ Rando, R. R., *Science*, (in the press).
- ² Scannell, J. P., Preuss, D. L., Demmy, T. L., Sello, L. H., Williams, T., and Stempel, A., *J. Antibiotics*, **25**, 122-127 (1972).
- ³ Jenkins, W. T., and D'Ari, L., *Biochem. biophys. Res. Comm.*, **22**, 376-382 (1966).
- ⁴ Bertland, L. H., and Kaplan, N. U., *Biochemistry*, **7**, 134-141 (1968).
- ⁵ Hammes, G. G., and Fasella, P., in *Chemical and Biological Aspects of Pyridoxal Catalysis*, (edit. by Snell, E. E., et al.) 185 (Macmillan & Co., New York, 1963).
- ⁶ Amador, E., and Wacker, W. E. C., *Clin Chem.*, **8**, 343-350 (1962).

Chelating agents for the binding of metal ions to macromolecules

POLYAMINOCARBOXYLATE chelating agents such as ethylenediaminetetraacetic acid (EDTA) form stable chelate complexes with the ions of many heavy metals. These metal ions exhibit a wide range of useful spectral and radioactive properties, such as electronic absorption, scattering of electrons and X rays, electron paramagnetic resonance spectra, the production of line-broadening and chemical shifts in nuclear magnetic resonance spectra, the emission of correlated gamma-ray cascades and various radioactive lifetimes and nuclear radiations. The preparation of chelating agents whose complexes can interact, in some selected manner, with biological macromolecules could make possible several new applications of metal ions as probes of biological systems.

The synthesis of 1-(*p*-aminophenyl)-EDTA (Fig. 1, R = NH₂) is a step toward this goal. In principle, its aromatic amino group can be acylated, alkylated or otherwise modified to form biologically active molecules or covalent labelling reagents. The specific interaction between a precursor of this compound, 1-(*p*-nitrophenyl)-EDTA (Fig. 1, R = NO₂), and an antibody molecule has been described previously¹.

We describe here the use of a derivative of the amino compound, 1-(*p*-benzenediazonium)-EDTA (Fig. 1, R = N₂⁺), as a reagent for labelling proteins. We also present data bearing on the usefulness of proteins labelled covalently with ¹¹¹In chelates as radiopharmaceuticals for the localisation of tumours.

The synthesis of 1-(*p*-aminophenyl)-EDTA (Fig. 1, R = NH₂) was accomplished in six steps, starting from benzaldehyde (details will be furnished on request). The amino compound then was diazotised² to form the chloride salt of 1-(*p*-benzenediazonium)-EDTA, or ⁺N₂Ph-EDTA (Fig. 1, R = N₂⁺).

The ⁺N₂Ph-EDTA was coupled to human serum albumin or to bovine fibrinogen (Blombäck fraction I-4 (ref. 3)) by reaction overnight at 4° C with 2% protein solutions in aqueous 0.01 M EDTA/0.12 M NaHCO₃ buffer, pH 8.1. The buffer ions and any

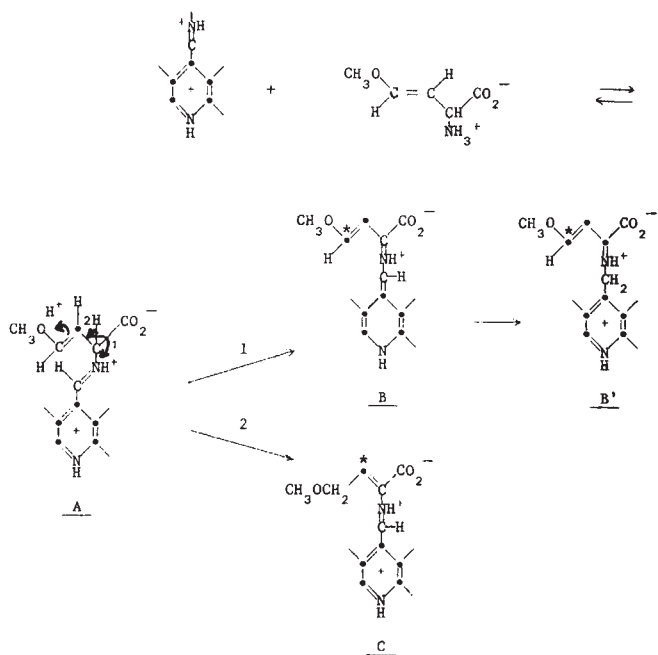


Fig. 4 Possible conversions of the catalytic product with AMB as substrate.

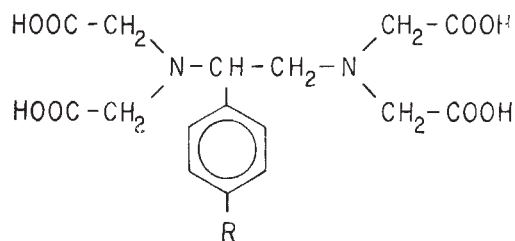


Fig. 1 The chelating agents discussed in the text.
R = NO₂, NH₂, or N₂⁺.

unbound reagent then were removed by extensive dialysis against heavy-metal-free 0.1 M citrate, pH 6. The presence of azotyrosine groups in the products was indicated by increased absorbance at 330 nm. Citrate buffer was chosen to facilitate the chelation of carrier-free ¹¹¹In ions by protein-bound -N₂Ph-EDTA groups. In this buffer, indium ions do not undergo hydrolysis or bind to native serum albumin or fibrinogen, but do bind readily to the conjugated proteins.

Indium-binding was studied experimentally by the technique of γ -ray perturbed angular correlations (PAC), using the ¹¹¹In ion as a radioactive probe of molecular motion¹. Based on the coincidence detection of γ rays emitted in cascade from a single nucleus, PAC depends on the rotational correlation time for fluctuating electric field gradients at the radioactive nucleus. The binding of ¹¹¹In ions to macromolecules is revealed by a change in the measured value of the integral perturbation factor, $\overline{G_{22}(\infty)}$. For carrier-free ¹¹¹InCl₃ in 0.1 M citrate at pH 6, $\overline{G_{22}(\infty)} = 0.60 \pm 0.01$, a value consistent with an indium complex of low molecular weight (in the same buffer, the NO₂Ph-EDTA chelate of ¹¹¹In yields $\overline{G_{22}(\infty)} = 0.58 \pm 0.02$). For a similar solution containing 1% bovine fibrinogen, $\overline{G_{22}(\infty)} = 0.57 \pm 0.02$, indicating little interaction between indium ions and the macromolecule. For 1% human serum albumin, $\overline{G_{22}(\infty)} = 0.59 \pm 0.02$. When 0.3 ml of a 2% solution of human serum albumin, which had been reacted with an equimolar amount of +N₂Ph-EDTA, was added to 0.3 ml of citrate buffer containing ¹¹¹InCl₃, binding was complete in less than 1 min. This was indicated by the perturbation factor; $\overline{G_{22}(\infty)} = 0.40 \pm 0.01$, a value which was not changed by further addition of the conjugated albumin. The addition of 1% bovine fibrinogen, which had been reacted with a two- to three-fold excess of +N₂Ph-EDTA, to ¹¹¹InCl₃ in citrate led to complete binding within several minutes; the equilibrium value of $\overline{G_{22}(\infty)}$ was 0.37 ± 0.02 .

Albumin and fibrinogen differ greatly in molecular size and shape; yet, the perturbation factors for ¹¹¹In bound to the two labelled proteins are quite similar. This suggests that in each case the link between chelate and macromolecule is flexible⁴.

We have determined the organ distribution and tumour uptake of radioactivity after injection of the ¹¹¹In-labelled proteins into specially prepared BALB/c mice. A tumour line, KHJJ, derived from a primary mammary carcinoma arising spontaneously in a mouse and maintained for over 100 transplant generations was used for the assay⁵. Three mice were injected with each compound, and the per cent dose per gram of organ was measured 24 h after injection. Results are given in Table 1, together with data obtained using commercial ¹³¹I-labelled human serum albumin in the same model system. These data suggest that proteins conjugated with -N₂Ph-EDTA chelates will be useful radiopharmaceuticals. The observed tumour:organ radioactivity ratios would be favourable for scanning studies on human patients, and the radioactive properties of ¹¹¹In are ideal for providing scans up to 1 week after administration with a minimum of radiation exposure to the patient^{6,7}. In addition, PAC studies can provide direct information on the stability *in vivo* of ¹¹¹In-labelled compounds⁸.

The mild reaction conditions and rapid metal-binding achieved here suggest that +N₂Ph-EDTA will be useful for labelling

Table 1 Distribution and uptake of labelled macromolecules in BALB/c mice with KHJJ Tumour

Organ	¹¹¹ In-albumin	¹¹¹ In-fibrinogen	¹³¹ I-albumin
Blood	4.6 ± 0.6	6.7 ± 1.1	6.8 ± 1.6
Lungs	3.4 ± 0.6	3.1 ± 0.3	3.4 ± 1.2
Liver	8.6 ± 0.3	9.2 ± 0.5	1.1 ± 0.3
Spleen	3.3 ± 0.1	7.0 ± 0.7	0.7 ± 0.2
Kidneys	11.4 ± 0.1	18.1 ± 1.9	2.4 ± 0.6
Tumour	9.7 ± 0.3	8.1 ± 0.8	3.3 ± 1.0
Muscle	0.8 ± 0.2	0.8 ± 0.2	0.9 ± 0.2
Bone	2.0 ± 0.4	1.9 ± 0.4	1.0 ± 0.5
Brain	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1
Skin	2.4 ± 0.4	2.0 ± 0.3	—

Each of the compounds listed was injected into three BALB/c mice which carried a KHJJ tumour (implanted in the flank 14 d before). The tumours were about 1 cm³ in volume. Twenty-four hours after injection, the mice were killed and samples of blood and major organs were taken, weighed and counted. Results are reported in terms of per cent total dose per gram organ, a measure of the concentration of radioisotope in the various tissues.

biologically active molecules with short-lived radioisotopes. Related chelating agents have potential applications to a broad range of problems. A full account of this work will be published elsewhere⁹.

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¹ Meares, C. F., Sundberg, M. W., and Baldeschwieler, J. D., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 3718-3722 (1972).

² Vogel, A. I., *Practical organic chemistry*, 567-568 (Longmans, Green, New York, 1948).

³ Blombäck, B., and Blombäck, M., *Ark. Kemi.*, **10**, 415-443 (1956).

⁴ Marshall, A. G., Werbelow, L. G., and Meares, C. F., *J. chem. Phys.*, **57**, 364-370 (1972). Erratum: page 4508.

⁵ Rockwell, S. C., Kallman, R. F., and Fajardo, L. F., *J. natn. Cancer Inst.*, **49**, 735-749 (1972).

⁶ Goodwin, D. A., Finston, R. A., Colombetti, L. G., Beaver, J. E., and Hupf, H., *J. nucl. Med.*, **10**, 337 (1969).

⁷ Hunter, W. W., and de Kock, H. W., *J. nucl. Med.*, **10**, 343 (1969).

⁸ Goodwin, D. A., Meares, C. F., and Song, C. H., *Radiology*, **105**, 699-702 (1972).

⁹ Meares, C. F., et al., *J. med. Chem.* (in the press).

Light-dependent phosphorylation of rhodopsin in living frogs

PHOSPHORYLATION of rhodopsin has been studied so far only *in vitro*. When suspensions of isolated and purified rod outer segments (ROS) from cattle and frogs have been mixed with γ -³²P-ATP and Mg²⁺, upon illumination ³²P-phosphate has been found to be bound covalently to rhodopsin in a slow dark reaction after bleaching. The activity of the water-

extractable kinase which mediates the phosphate transfer was found to be independent of light, but rhodopsin was not acceptable as a substrate until it had been bleached by light⁴. The slow rate at which phosphorylation occurs (half time 3–5 min (refs 1–4)) suggests that it may be involved in light adaptation of the rods which also seems to be a slow process⁵.

If the phosphorylation reaction plays a role in vision, it should be reversible; rhodopsin should undergo a cycle of phosphorylation and dephosphorylation. This important question, however, could not be clarified by the *in vitro* experiments: in some instances, dephosphorylation could be observed to occur to some extent^{2,3}, but mostly, the phosphorylation seemed to be irreversible^{1,4,8}. If there is capacity for dephosphorylation, it seems to be easily lost in the system of isolated ROS. Therefore the reaction had to be studied in living animals. That protein is phosphorylated in the ROS of living frogs was reported by Hall and Bacharach⁷ as early as 1970, but they were not interested in the light dependency of the effect. My experiments show that phosphorylation and dephosphorylation of rhodopsin are physiological reactions in the photoreceptors of the frog.

³²P-inorganic phosphate, in two portions of about 2 mCi each, was injected into the dorsal lymphatic sac of frogs (*Rana esculenta*, body weight 40–50 g) at an interval of 15 h. Each injection solution contained 20 μ mol of phosphate in 0.3 ml. The frogs were maintained individually in glass jars containing water, at 23°C in the dark. During the full incubation period of 41 h they excreted on the average $7.5 \pm 4\%$ (s. d.) of the total injected ³²P into the water, but

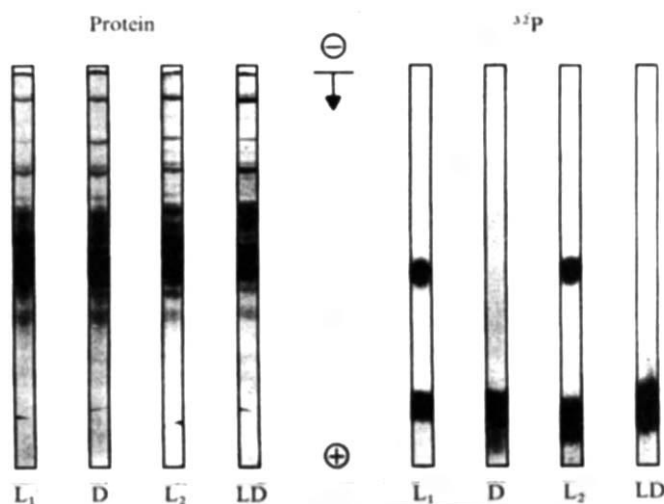


Fig. 1 Electrophoresis of ROS preparations obtained from four different frogs, on gels (length 11 cm) containing 5.8% polyacrylamide and 1% sodium dodecylsulphate (SDS) (ref. 9). To isolate the ROS, both retinas of each frog were shaken vigorously, using a Heidolph Reax I mixer, for 60 s in 2 ml of 45% sucrose solution containing 5 mM Tris-acetate (pH 7.3), 65 mM NaCl, and 1 mM MgCl₂. Differential centrifugation was carried out according to McConnell⁸. The final ROS pellet was solubilised with 100 μ l of 2% SDS containing 2% 2-mercaptoethanol, for 20 min at 45°C. Insoluble material derived from pigment epithelium was removed by centrifugation. L₁, Light-adapted frog, with retinas and ROS prepared in white light; L₂, light adapted with retinas and ROS prepared in dim red light; D, dark adapted for 2 d; LD, light adapted and then dark adapted for 75 min. The three light-adapted frogs were exposed for 20 min to diffuse white light of 450 lx. On the left side, photographs of gels stained with Coomassie brilliant blue are shown; the corresponding autoradiographs, exposed for 4 d on X-ray film, are shown on the right. The ink mark near the lower end of the stained gels indicates the position of the marker dye, pyronin Y, which ran to about the same position as the phospholipids. The autoradiographed gels contain 2–5 times more ROS material than the stained gels.

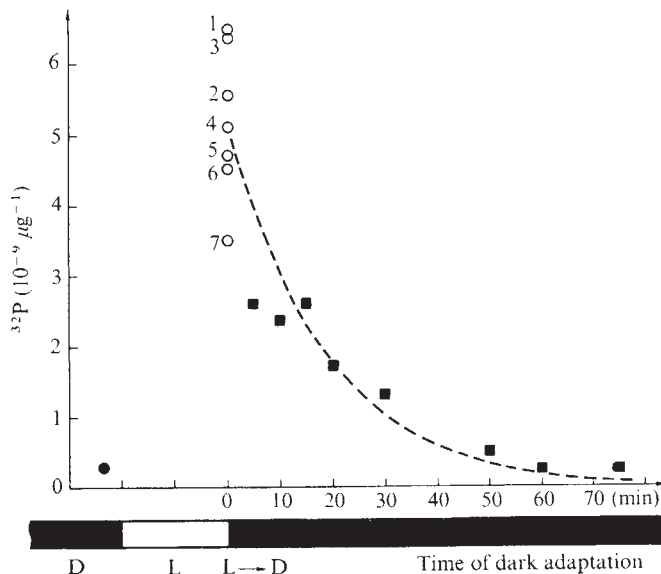


Fig. 2 ³²P bound to rhodopsin as a function of light/dark adaptation of 16 frogs. ●, Dark adapted (D), for 2 d; ○, light adapted (L) for 20 min; ■, light adapted for 20 min and then dark adapted for the times indicated in the abscissa. The seven light-adapted frogs are numbered as 1–7. ---, A theoretical first order decay curve based on a half time of 13 min, starting from the average phosphorylation level of the seven light-adapted frogs. The radioactivity was determined by scintillation counting of gel slices⁴. The amount of rhodopsin was determined from the area of the rhodopsin peak in stained and scanned gels; a calibration curve was obtained with 40 stained and scanned gels containing different amounts of purified cattle rhodopsin of known concentration. The staining and destaining procedure⁹ was carried out in strictly standardised conditions, and it was assumed that cattle and frog rhodopsin both have the same staining properties with Coomassie brilliant blue. The amount of rhodopsin isolated in the ROS was in general about 100 μ g per frog but varied from 150–40 μ g. The ³²P activity was related to the amount of rhodopsin, and to the total ³²P in each frog body at the moment of its death (total injected d.p.m. minus total excreted c.p.m. during the incubation). In a typical light-adapted frog, for example frog No. 2, there were 5,260 d.p.m. of ³²P bound to 146 μ g of rhodopsin, and 11,200 d.p.m. bound to phospholipid. 7×10^9 d.p.m. had been injected into the frog, 7% of which it had excreted during the incubation. Thus the value plotted on the ordinate is $5,260/146 \times (7-0.5) \times 10^9$ d.p.m./ μ g $\times$ d.p.m. = 5.5×10^{-9} μ g⁻¹.

this value was as high as 13 and 14% in some frogs and as low as 0.5% in another. Forty to forty-one hours after the first injection, the frogs were subjected to different light/dark conditions: some were left in the dark, others were light adapted, and others first light and then dark adapted. Light adaptation was accomplished in all cases by exposing the frogs for 20 min to white diffuse light (450 lx) from a fluorescent lamp. At the end of the light/dark treatment (41 h after the first injection) the frogs were killed and the retinas were dissected in dim red light. The two retinas of a frog contained on the average 10^6 decompositions per minute (d.p.m.) of ³²P. The ROS were shaken off and purified by a modification of McConnell's procedure⁸ (Fig. 1). To minimise enzymatic reactions during the preparation, all operations were done at 0°C as fast as possible and, unless otherwise stated, in dim red light. The elapsed time between killing a frog and solubilising its ROS was 35–40 min.

The solubilised ROS were separated by SDS gel electrophoresis. Results obtained from four different frogs are shown in Fig. 1. The purity of the ROS preparation is indicated by the relatively large rhodopsin band in the stained gels shown at the left, demonstrating that rhodopsin is the predominant protein¹⁰. The two radioactive bands in the autoradiographed gels (Fig. 1, right)

correspond to ^{32}P -rhodopsin (middle of gells) and ^{32}P -phospholipids (lower end). The phospholipid nature of the lower band was demonstrated by extracting the ROS with chloroform/methanol¹¹ followed by thin layer chromatographic analysis of the extract. This phospholipid band was found in all frogs, independent of their light/dark treatment, and represents freshly synthesised phospholipids which were incorporated into the ROS during the 41 h incubation period. Inorganic phosphate was also found when the gels were electrophoresed for shorter times, but normally most of the phosphate was eluted into the anode buffer because of its faster mobility, compared to the phospholipids.

The rhodopsin obtained from the light-adapted frogs was phosphorylated (Fig. 1, L_1 and L_2), but that from the dark-adapted frogs was not. One of the dark-adapted frogs (Fig. 1, D) was dark-adapted for 2 d before being killed and the other (LD) was dark-adapted for only 75 min following an illumination period of 20 min; ^{32}P was not bound to the rhodopsin of either animal. This indicates that rhodopsin was phosphorylated when the frogs were exposed to light and dephosphorylated during dark adaptation.

Quantitative analyses indicate that phosphorylation of rhodopsin in the light adapted frogs was about 20 times that in the completely dark-adapted frogs (Fig. 2). The average ^{32}P bound to the rhodopsin of the seven light-adapted frogs was 5.1×10^{-8} per μg of rhodopsin (s. d.: $\pm 1.1 \times 10^{-9} \mu\text{g}^{-1}$), compared to $0.25 \times 10^{-8} \mu\text{g}^{-1}$ in completely dark-adapted frogs. No significant difference in the final yield of ^{32}P -rhodopsin could be observed between retinas and ROS from light-adapted frogs prepared in white light (Fig. 2, frogs 1, 4, 6, and 7) and those prepared in dim red light (Fig. 2, frogs 2, 3 and 5). This indicates that no significant, light-dependent changes in the phosphorylation level occurred during the 40 min preparation period at 0°C .

In the eight frogs which were dark adapted after a 20 min illumination period, the phosphorylation level of rhodopsin decreased slowly with increasing time in the dark, and after approximately 1 h the dephosphorylation was complete. If the dephosphorylation kinetics were first order, its half time would be about 13 min (Fig. 2, dotted line); but quantitative kinetic analysis is not possible because of animal to animal variations and the small number of animals used.

The slow rate of dephosphorylation shown in Fig. 2 is comparable to published rates of dark adaptation measured by psychophysical^{12,13} and electrophysiological^{14,15} methods. Kohlrausch¹² has shown that complete recovery of psychophysical sensitivity during dark adaptation in the human eye takes about 1 h. Similarly, electrophysiological studies on isolated retinas of frog¹⁴ and *Necturus*¹⁵ show that, following extensive bleaching, recovery of sensitivity occurs within about an hour.

Evidence has increased in the past few years that, besides 'neural' control mechanisms, substantial parts of dark adaptation take place in the photoreceptors themselves¹⁴⁻¹⁷. Rushton¹⁶ has demonstrated by combined psychophysical and spectrophotometric measurements that during slow dark adaptation, recovery of the logarithm of sensitivity occurs exactly in parallel with photopigment regeneration. On the other hand, electrophysiological experiments^{14,17} on isolated retinas in which regeneration of rhodopsin is believed to be negligible, demonstrated that slow recovery of sensitivity by 2-4 decades occurs at the receptor level even though regeneration is minimal. Thus there must be mechanism(s) of slow dark adaptation at the receptor level which are independent of photopigment regeneration. My experiments give evidence of a chemical reaction, namely dephosphorylation of phosphorylated rhodopsin, which takes place in the photoreceptors under the conditions and at about the rate of dark adaptation. This dephosphorylation seems to be independent of photopigment regeneration,

as recent experiments with isolated frog retinas have indicated (H.K., and Bader, unpublished). We have also shown⁴ that the regeneration of rhodopsin *in vitro* is not affected by the binding of phosphate to opsin, nor is the binding affected by regeneration; phosphorylated opsin regenerated with 11-*cis* retinal to form phosphorylated rhodopsin, and gave as good a result as in the nonphosphorylated control experiment, without any loss of phosphate during the regeneration.

The molecular mechanism by which this phosphorylation reaction regulates the sensitivity of the rods remains to be elucidated. As suggested earlier⁴, the phosphate group bound to rhodopsin and/or opsin in the light adapted state could alter the permeability of the membrane in which it is located and thus regulate ion fluxes through adjacent pores. This membrane could be the disk as well as the cell plasma membrane which has also been shown to contain rhodopsin¹⁸. Phosphorylation could also influence the binding of Ca^{2+} to the photoreceptor membranes. It is likely that during dark adaptation the phosphate is cleaved from rhodopsin by the action of a phosphatase.

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- ¹ Kühn, H., and Dreyer, W. J., *FEBS Lett.*, **20**, 1-6 (1972).
- ² Bownds, D., Dawes, J., Miller, J., and Stahlman, M., *Nature new Biol.*, **237**, 125-127 (1972).
- ³ Frank, R. N., Cavanagh, H. D., and Kenyon, K. R., *J. biol. Chem.*, **248**, 596-609 (1973).
- ⁴ Kühn, H., Cook, J. H., and Dreyer, W. J., *Biochemistry*, **12**, 2495-2502 (1973).
- ⁵ Dowling, J. E., and Ripps, H., *J. gen. Physiol.*, **60**, 698-719 (1972).
- ⁶ Bownds, D., Dawes, J., and Miller, J., in *Biochemistry and Physiology of Visual Pigments* (edit. by Langer, H.), 267-273 (Springer-Verlag, Berlin, Heidelberg, New York, 1973).
- ⁷ Hall, M. O., and Bacharach, A. D. E., *Nature*, **225**, 637-638 (1970).
- ⁸ McConnell, D. G., *J. Cell Biol.*, **27**, 459-473 (1965).
- ⁹ Fairbanks, G., Steck, T. L., and Wallach, D. F. H., *Biochemistry*, **10**, 2606-2617 (1971).
- ¹⁰ Bownds, D., Gordon-Walker, A., Gaide-Huguenin, A. C., and Robinson, W., *J. gen. Physiol.*, **58**, 225-237 (1971).
- ¹¹ Bligh, E. G. and Dyer, W. J., *Can. J. Biochem. Physiol.*, **37**, 911-917 (1959).
- ¹² Kohlrausch, A., *Handb. Norm. Pathol. Physiol.*, **12/2**, 1499-1594 (Springer-Verlag, Berlin, 1931).
- ¹³ Rushton, W. A. H., *J. Physiol., Lond.*, **156**, 193-205 (1961).
- ¹⁴ Hood, D. C., Hock, P. A., and Grover, B. G., *Vision Res.*, **13**, 1953-1963 (1973).
- ¹⁵ Norman, R. A., and Werblin, F. S., *J. gen. Physiol.*, **63**, 37-61 (1974).
- ¹⁶ Rushton, W. A. H., and Powell, D. S., *Vision Res.*, **12**, 1073-1081 (1972).
- ¹⁷ Grabowski, S. R., Pinto, L. H., and Pak, W. L., *Science*, **176**, 1240-1242 (1972).
- ¹⁸ Jan, L. Y., and Revel, J. P., *J. Cell Biol.* (in the press).

Regulation of arginine catabolism in *Aspergillus nidulans*

IN *Aspergillus nidulans*, mutations at more than 20 loci result in increased levels of two arginine catabolic enzymes, arginase and ornithine δ -transaminase (OTase)¹. The large number of genes involved either directly or indirectly in regulation of arginine catabolism makes interpretation of the regulatory mechanism(s) complicated. Some of these genes at least are concerned with catabolite repression rather than with control of specific induction and repression processes². Here we

present evidence that the synthesis of arginase and OTase in *A. nidulans* is regulated in a positive fashion. Interrelations between the gene responsible for positive control and several other genes involved in the regulation of these enzymes were established. This enabled us to propose a model of the regulatory mechanism involving interaction between a specific positive and a nonspecific negative mode of regulation and providing a possible explanation of ammonium repression.

In *A. nidulans* arginine can serve as a source of proline both in the wild-type strain and in proline mutants blocked in the first two steps of the main pathway of proline synthesis. Use of arginine for proline synthesis depends on the presence and on the inducibility of arginine catabolic enzymes, arginase and OTase. Mutants with derepressed arginase and OTase were obtained in *A. nidulans* as suppressors of proline mutations^{3,4}. A mutant designated *arcA*^d 47 (former symbol *suG* 47 *pro*) (ref. 2) shows approximately 10-fold higher levels of both enzymes compared with those in the wild-type strain (Table 1). In heterokaryons and diploids *arcA*^d 47 is semidominant over the wild-type allele. By means of the haploidisation technique⁵ the *arcA* locus was assigned to linkage group VIII. The *arcA* locus is not linked to the structural genes for arginase and OTase.

We selected for mutants unable to use arginine as a source of proline and isolated mutants in the arginase and OTase structural genes, mutants defective in arginine transport (unpublished data), mutants hypersensitive to catabolite repression² and mutants designated by the *arcA*^r symbol. In *arcA*^r1 mutant the uninduced levels of arginase and OTase are the same as in the wild type, but neither enzyme is inducible by exogenous arginine. On the basis of recombination tests the *arcA*^r and *arcA*^d mutations seemed likely to be located within the same gene as no *arcA*⁺ recombinants were found among 600 progeny of the cross *arcA*^r × *arcA*^d. This assumption was strengthened by the results of studies on the ultraviolet-induced revertants of the *arcA*^r1 mutant. One of the revertants was found to exhibit all properties of the *arcA*^d47 mutant. By crossing this revertant to the wild type and *arcA*^d47 strains it was established that the reversion was due to mutation in or very close to the *arcA* locus. The *arcA*^r1 mutation is recessive to the *arcA*^d47 and *arcA*⁺ alleles.

The simplest interpretation of the properties of the *arcA*^r and *arcA*^d mutants is that the *arcA* gene specifies the product (inducer) which is necessary for expression of the arginase and OTase structural genes. In the wild type the inducer requires activation by the coinducer, arginine, whereas in the *arcA*^d47 mutant the inducer is active even in the absence of arginine. In the *arcA*^r1 mutant the inducer is inactive or it is not produced.

Table 1 Arginase and OTase activities in various mutants

No. Mutant*	Enzyme activity†			
	Arginase MM	OTase MM	MM + arg	MM + arg + NH ₄
(1) Wild type	0.20	7	110	30
(2) <i>arcA</i> ^r 1	0.20	5	5	5
(3) <i>arcA</i> ^d 47	4.0	50	105	95
(4) <i>suA25pro</i>	2.0	70	130	120
(5) <i>suD25pro</i>	2.0	75	140	130
(6) <i>arcA</i> ^r 1 <i>suA25pro</i>	0.20	10	22	—
(7) <i>arcA</i> ^r 1 <i>suD25pro</i>	0.15	6	7	—
(8) <i>arcA</i> ^d 47 <i>suA25pro</i>	4.0	180	190	—
(9) <i>arcA</i> ^d 47 <i>suD25pro</i>	4.0	200	200	—

Mycelia for enzyme assays were grown on standard minimal medium (MM) (10 g glucose l⁻¹ as carbon source, 10 mM sodium nitrate as nitrogen source) and transferred when necessary to media containing 5 mM arginine or 5 mM arginine and 5 mM ammonium tartrate for 6 h.

* Genotypes of mutants: nos 1–4—*proA6 pabaA9 biA1*; nos 5, 7, 9—*proA6 adF9 y; phenA2*; nos 6, 8—*proA6 pabaA9 biA1; phenA2*.

† Units of arginase and OTase activity are that amount of enzyme which forms 1 μmol of urea and 1 μmol of glutamic γ-semialdehyde per min per mg protein respectively.

Arginase and OTase synthesis in *A. nidulans* is subject to both glucose and nitrogen catabolite repression. The mechanism of catabolite repression in fungi is not yet fully understood, but ammonium seems to be involved in nitrogen catabolite repression.⁶ One of the effects of ammonium is the prevention of induction of arginase and OTase by arginine⁶. Similar effects of ammonium on arginase and OTase induction were observed in yeasts^{7,8} and on induction of several other catabolic enzymes in *A. nidulans* and *Neurospora crassa*⁹.

The arginase and OTase levels in the *arcA*^d47 mutant are not fully derepressed and the synthesis of both enzymes can still be induced by exogenous arginine (data for OTase are given in Table 1). In contrast to the wild type, the presence of ammonium in the culture medium does not prevent induction of arginase and OTase in the *arcA*^d47 mutant. Assuming that the function of the *arcA* gene is interpreted correctly, it can be concluded that ammonium interferes with the process of activation of the *arcA* gene product (inducer) by arginine. As a result of the *arcA*^d47 mutation the inducer is able to function in the absence of arginine (although it retains some affinity for arginine) and at the same time becomes insensitive to the effects of ammonium.

Apart from the *arcA*^d47 mutation, mutations in six *supro* loci (designated respectively *A*, *D*, *E*, *H*, *J* and *L*) result in derepression of arginase and OTase to the level exceeding

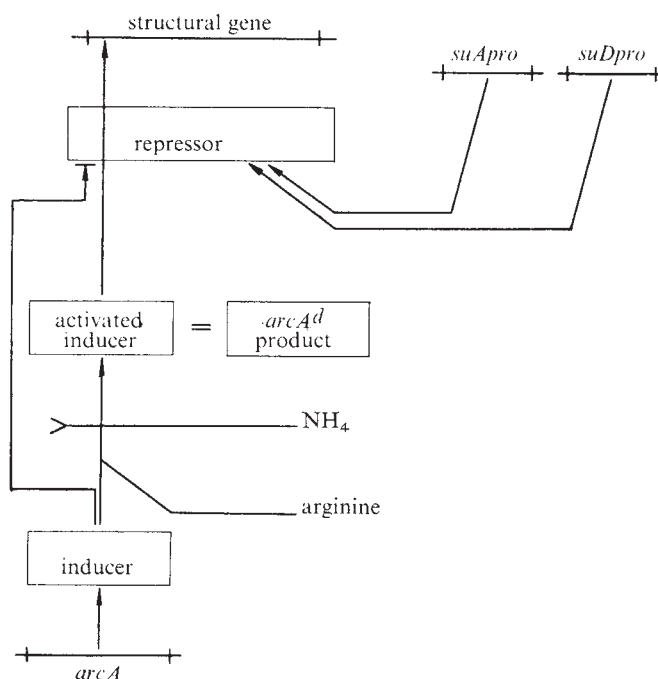


Fig. 1 Regulation of arginine catabolism.

approximately 10-fold the basal level of these enzymes observed in the wild-type strain¹. The levels of both enzymes are not repressed when *supro* mutants are grown in the presence of ammonium. All 25 mutations at these loci which we have studied are recessive and none of the loci is closely linked to any other or to the loci of the arginase and OTase structural genes. Two mutants, *suA25pro* and *suD25pro* were chosen and crossed to the *arcA*^d47 and *arcA*^r1 strains. The appropriate double mutants were selected from the progeny of these crosses and assayed for arginase and OTase activity. The results given in Table 1 can be summarised as follows: (1) The effects of *suA25pro* and *arcA*^d47 mutations are additive, as are the effects of *suD25pro* and *arcA*^d47. The level of both enzymes in double mutants corresponds to their maximal level observed in the wild type grown in the presence of arginine. (2) The *arcA*^r1 mutation cancels the effects of both *suA25pro* and *suD25pro* mutations and makes arginase and OTase

noninducible by arginine in *suA* and *suD* strains. The *arcA*¹ mutation is thus fully epistatic to the two suppressor mutations studied.

It can be concluded that the *suApro* and *suDpro* genes participate in the formation of a repressor which maintains the arginase and OTase synthesis at the low level observed in the wild-type strain grown in the absence of arginine. The function of the repressor is abolished by the activated inducer specified by the *arcA* gene. Activation of the inducer is necessary only to overcome the effects of repression exerted by the products of *suApro*, *suDpro* and possibly other *supro* genes: when the repressor is not active as in *suA25pro* and *suD25pro* mutants, the synthesis of arginase and OTase is derepressed despite the absence of arginine. On the other hand, inactivation of repressor alone is not sufficient to cause derepression of enzyme synthesis. Low levels of arginase and OTase in *arcA*¹ *suA25pro* and in *arcA*¹ *suD25pro* double mutants indicate that the presence of the normal product of the *arcA* gene is obligatory for derepression to occur. All above considerations are summarised in Fig. 1.

We have no information concerning the nature of the *suApro* and *suDpro* gene products. Thus we do not claim that these genes participate in formation of repressor equivalent to that of the *Escherichia coli* lactose system. This would be premature, especially knowing that in its formation not only *suApro* and *suDpro* but probably also four other genes are involved. Moreover, there is some information that mutations in these genes, in addition to controlling arginine catabolism, affect some other systems; for example they cause derepression of nitrate reductase synthesis (unpublished results). It was also found that the *suD19pro* mutation affects the synthesis of ornithine transcarbamylase¹⁰ and cancels certain effects of mutations in the *areA* gene which controls use of a variety of nitrogen sources⁹. It seems therefore that if *suDpro* and other genes of this group are responsible for formation of repressor of some kind, this repressor is highly nonspecific.

As pointed out by Gross¹¹, in fungi the positive rather than negative control of gene expression is the rule. The only exception so far was found by Wiame and his coworkers⁸ who proved the existence of operator genes in the arginine system in yeasts. The universality of positive regulation suggests that in fungi general regulatory mechanisms (those which control expression of genes concerned with many different enzymatic systems) can operate at the level of activation of inducers specific for structural genes of a single system. Such an interpretation was used in this paper to explain the effects of ammonium on the synthesis of arginine catabolic enzymes. We do not postulate that this is the only way in which ammonium affects the synthesis of catabolic enzymes. For example, ammonium also seems to participate in the mechanism responsible for maintaining the basal level of several enzymes^{2,6,12,13}. It is also probable that the inhibitory effect of ammonium upon induction of catabolic enzymes is mediated by the product(s) of particular gene(s), possibly including the *areA* gene which has recently been studied in several laboratories (see ref. 9).

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¹ Klimczuk, J., and Weglenski, P., *Acta microbiol. pol.*, **A6**, 93-100 (1974).

² Bartnik, E., Guzewska, J., and Weglenski, P., *Molec. gen. Genet.*, **126**, 85-92 (1973).

³ Weglenski, P., *Genet. Res.*, **8**, 311-321 (1966).

⁴ Weglenski, P., *J. gen. Microbiol.*, **47**, 77-85 (1967).

⁵ McCully, K. S., and Forbes, E., *Genet. Res.*, **6**, 352-359 (1965).

⁶ Bartnik, E., Piotrowska, M., and Weglenski, P., *Molec. gen. Genet.*, **126**, 75-84 (1973).

⁷ Middelhoven, W. J., *Antonie van Leeuwenhoek*, **36**, 1-19 (1970).

⁸ Wiame, J. M., *Curr. Top. Cell. Regulation*, **4**, 1-38 (1971).

⁹ Arst, H. N., jun., and Cove, D. J., *Molec. gen. Genet.*, **126**, 111-141 (1973).

¹⁰ Cybis, J., Piotrowska, M., and Weglenski, P., *Molec. gen. Genet.*, **118**, 272-277 (1972).

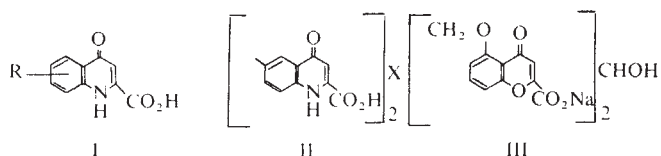
¹¹ Gross, S. R., *A. Rev. Genet.*, **3**, 395-424 (1969).

¹² Hynes, M. J., *J. Bact.*, **103**, 482-487 (1970).

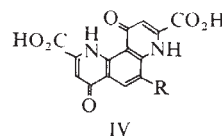
¹³ Pateman, J. A., Kinghorn, J. R., Dunn, E., and Forbes, E., *J. Bact.*, **114**, 943-950 (1973).

Inhibition of allergic reactions by a novel phenanthroline ICI 74,917

FOR 4 yr we have been investigating the biological properties of a series of kynurenic acids (I) and bridged analogues (II), related to the chromone carboxylic acids, of which disodium cromoglycate (III) is an example. Some of the quinolone acids I and II were found to be as effective as disodium



cromoglycate in inhibiting passive cutaneous anaphylaxis (PCA) in rats^{1,2}. Compounds of much greater activity in PCA were however discovered among a series of tricyclic quinolone acids, the 2,8-dicarboxy-4,10-dioxo-1,4,7,10-tetrahydrophenanthrolines (IV)³. Here we describe the properties of one of the more active of these compounds, ICI 74,917 (IV, R = *n*-butyl). It can be prepared by the reaction of 1,3-diamino-4-*n*-butyl benzene with oxaloacetic ester and thermal cyclisation of the bis-anil to the phenanthroline-2,8



dicarboxylate which is then hydrolysed to ICI 74,917. It melts at 300° C (decomposition) and is insoluble in most organic solvents although its alkali metal salts are soluble in water. It may exist in tautomeric forms other than that indicated.

Administered intravenously as the sodium salt, at the time of antigenic challenge, ICI 74,917 inhibited PCA provoked by reaginic (IgE-like) antibody prepared according to Mota⁴ against egg albumin or according to Ogilvie⁵ against the nematode parasite *Nippostrongylus brasiliensis*. A comparison of the ID₅₀ (the dose required to produce a 50% inhibition of PCA) of each compound revealed that ICI 74,917 was some 300 times more active than disodium cromoglycate (Table 1).

In vitro, ICI 74,917 was not an antagonist of histamine or 5-hydroxytryptamine. When administered intravenously, it did not inhibit blueing reactions provoked in the normal rat by intradermal injections of histamine, 5-hydroxytryptamine or Compound 48/80 but PCA reactions induced in this species by heat-stable (IgG) homocytotropic and heterologous (guinea pig and rabbit) antibodies were significantly and consistently inhibited to a slight extent (30%).

It seemed possible, on the basis of these findings that ICI 74,917 might prevent the release of inflammatory medi-

ators from mast cells which follows interaction of antibody and antigen. This hypothesis was supported by the demonstration that ICI 74,917, when present at antigenic challenge at concentrations between 10^{-8} M and 10^{-6} M, reduced significantly the amount of histamine liberated from rat peritoneal mast cells sensitised *in vitro* with reaginic antibody.

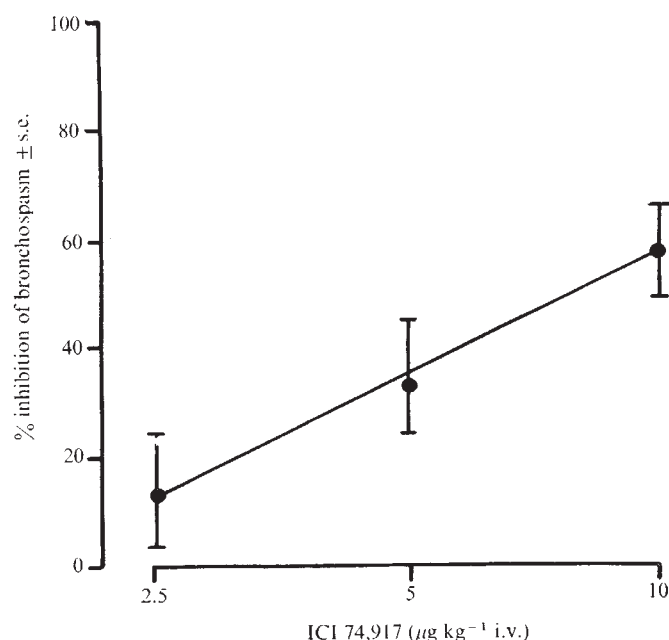


Fig. 1 Inhibition of allergic bronchospasm in the guinea pig by ICI 74,917. Groups of 10 animals were passively sensitised with guinea pig anti-egg albumin serum and challenged 18 h later with antigen (1 mg), and with saline or varying doses of ICI 74,917 in saline. Bronchospasm was assessed by the Konzett-Rössler technique as modified by Davies and Johnston⁷. Results are expressed as % inhibition of bronchospasm ($\pm$ s.e.) 4 min after antigenic challenge compared with sensitised, control animals given antigen and saline alone.

ICI 74,917 also possesses anti-allergic activity in other species. In the mouse, PCA induced by a reaginic antibody prepared according to Mota⁶ was inhibited, but the amount (0.5–2.5 mg kg⁻¹ intravenously (i.v.)) of compound required to achieve a regular dose dependent inhibition (27 to 67%) was higher than that required in the rat. Although there was no evidence of bronchodilator activity in the guinea pig, the compound, administered intravenously, conferred partial protection against systemic anaphylaxis and at doses comparable to those necessary to inhibit PCA in the rat, reduced in a dose-dependent manner allergic bronchospasm (Fig. 1) in guinea pigs passively sensitised with an homologous nonreaginic antibody prepared according to Davies *et al.*⁷. On the other hand, ICI 74,917, even at relatively high doses, did not inhibit PCA induced with the same antibody, suggesting in this species, some tissue specificity for the compound. Disodium cromoglycate showed no activity in these models in the mouse or guinea pig at doses up to 50 mg kg⁻¹ i.v..

Table 1 A comparison of ICI 74,917 and disodium cromoglycate on reagent-mediated PCA

Compound	Antigen-antibody system	ID ₅₀ (mg kg ⁻¹ i.v. $\pm$ 95% confidence limits)	Relative potency (95% confidence limits)
Disodium cromoglycate	Egg albumin	2.8 $\pm$ 0.2	1.0
	<i>N. brasiliensis</i>	3.4 $\pm$ 0.2	1.0
ICI 74,917	Egg albumin	0.009 $\pm$ 0.0005	323 $\pm$ 30
	<i>N. brasiliensis</i>	0.01 $\pm$ 0.0006	308 $\pm$ 24

These studies have shown that ICI 74,917 exhibits anti-allergic properties additional to those possessed by disodium cromoglycate in that it is effective in laboratory species other than the rat. ICI 74,917 may therefore be of value in the treatment of allergic conditions in man.

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¹ Evans, D. P., Gilman, D. J., O'Mant, D. M., and Thomson, D. S., *UK Patent 1,334,705*.

² Waring, W. S., *UK Patent 1,305,820*.

³ Waring, W. S., *UK Patent 1,308,787*.

⁴ Mota, I., *Immunology*, **7**, 681–699 (1964).

⁵ Ogilvie, B. M., *Immunology*, **12**, 113–131 (1967).

⁶ Mota, I., *Immunology*, **12**, 343–348 (1967).

⁷ Davies, G. E., and Johnston, T. P., *Int. Archs Allergy*, **41**, 648–654 (1971).

Regulation of clonal development of immune responding cells by antibody of maternal origin

PASSIVELY administered antibody can regulate homologous antibody production in adult animals but the mechanisms are not completely understood¹. Maternal antibody transmitted to the foetus also has such an effect². This effect is usually transient and reversible; animals regain the ability to respond to antigen after passively administered antibody is removed^{3,4}. Adoptively transferred spleen cells from donor animals previously exposed to antibody are also able to respond normally⁵. Mature B and T cells seem also not to be affected by exposure to passively administered antibody^{6,7}.

It is not known if passively administered antibody has any influence upon the clonal development of immune cells. So, we studied the effect of the administration of specific maternal antibody on the ability of progeny to mount antibodies against the appropriate antigen. This was undertaken because in certain species the progeny are exposed to maternal antibody *in utero* during the course of foetal clonal development of immune cells⁸. We found that clonal cells responding to at least some antigens may proliferate as a consequence of being exposed to homologous antibody during foetal or neonatal life.

Female C₃H/He mice were immunised several times with sheep red blood cells (SRBC) before mating. The progeny,

Table 1 Plaque-forming ability of normal and SRBC mice to SRBC in adoptive transfer experiments

Donor spleen	No. of mice	Average PFC in recipient spleens per 10 ⁶ donor spleen cells $\pm$ s.e.
Normal mice	10	538 $\pm$ 344
SRBC mice*	11	506 $\pm$ 291

Spleen cells of 3-week-old normal or SRBC mice were transferred to syngeneic normal X-irradiated mice. Recipient mice were immunised intravenously with 4×10^8 SRBC 8 d before being assayed for their splenic PFC response. PFC to SRBC was assayed as described⁹. PFC in recipients that received either no antigen or no donor cells were negligible.

* Progeny mice whose mother had received SRBC immunisation several times before mating.

SRBC mice, were bled at 3 weeks of age and spleen cells were transferred to normal syngeneic X-irradiated mice. The numbers of plaque-forming cells (PFC) to SRBC amongst the recipient spleen cells were assayed 8 d after antigen injection. Spleen cells from progeny of nonimmune mothers were treated and tested in the same way as a control.

It was found that the number of PFC to SRBC amongst the spleen cells of recipient mice were similar whether recipients received donor spleen cells from normal or SRBC mice (Table 1). At the time of cell transfer, SRBC mice had a high titre of anti-SRBC antibody in their serum which should have been sufficient to suppress active production of antibody to SRBC. These results suggest that maternal antibody against SRBC does not affect the foetal development of specific immune responding cells.

When similar experiments were done using DNA as the test antigen different results were obtained. Female C₃H/He mice were immunised several times with denatured calf thymus DNA to attain high titres of anti-DNA antibody. The progeny, DNA mice, from immunised mothers were then treated in the same way as SRBC mice substituting DNA as the specific antigen. The numbers of PFC to calf thymus DNA amongst spleen cells from recipients who received spleen cells from DNA mice were three to four times those of the controls (Table 2). Although the number

Table 2 Plaque-forming ability of normal and DNA mice to DNA in adoptive transfer experiments

Donor spleen	No. of mice	Average PFC in recipient spleens per 10 ⁶ donor spleen cells $\pm$ s.e.	
		Exp. 1	Exp. 2
Normal mice	3	40.1 $\pm$ 2.7	41.2 $\pm$ 12.9
DNA mice*	3	117.9 $\pm$ 19.0	161.7 $\pm$ 37.7

Experimental procedures are essentially the same as those in Table 1. Heat-denatured calf thymus DNA was conjugated with equal amounts of methylated bovine serum albumin and emulsified with complete Freund's adjuvant. Recipient mice were challenged to a single injection of antigen (100 μ g as DNA) subcutaneously. Anti-DNA PFC were assayed using SRBC coupled with DNA by chromium chloride¹¹. PFC in recipients that received either no antigen or no donor cells were negligible.

* Progeny mice whose mother had received DNA immunisation several times before mating.

of animals studied and the number of antibody-producing cells were small, the differences were significant.

Another experiment also showed the increased PFC response to DNA in DNA mice. Maternal anti-DNA antibody in DNA-mice became undetectable by 6 weeks of age. Normal and DNA mice were challenged with DNA at this age and the PFC response in their spleens was compared 4 d after DNA injection. As shown in Table 3, PFC in the spleen cells of DNA mice were much more than those of control mice.

The enhanced ability of DNA mice to respond to DNA is probably mediated by passively transferred maternal antibody, although the possible involvement of other factors such as passively transferred antigen, carrier protein, and adjuvant were not considered in these experiments. The observed effect of anti-DNA antibody (presumably maternal) in the progeny seems to be different from the suppressive effect of antibody on active antibody production in adult mice, as the enhanced ability to respond to DNA in DNA mice remained even after removal of antibody.

Increased PFC response to DNA in DNA mice may be interpreted as a result of maternal anti-DNA antibody influencing the clonal development of DNA-specific immune responding cells in the progeny. We do not know to what extent these observed effects of maternal antibody are

Table 3 PFC response in normal and DNA mice challenged to DNA

Group	No. of mice	Average PFC per spleen $\pm$ s.e.
Normal mice	7	1,644 $\pm$ 750
DNA mice	6	4,642 $\pm$ 2,111

Six-week-old normal and DNA mice were challenged to a single injection of DNA and were assayed for their splenic PFC response 4 d after antigen injection.

general. Antigen selectivity clearly exists. DNA is a weak antigen, probably because of its self-antigen nature¹⁰. The clonal development of immune responding cells to DNA may be suppressed during ontogeny of the immune systems, and this may in some way be modified by maternal anti-DNA antibodies. Antigen selectivity and the mechanism of this phenomenon need further clarification. A detailed paper will be published separately.

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- Uhr, J. W., and Möller, G., *Adv. Immun.*, **8**, 81-127 (1968).
- Solomon, J. B., Rindell, G. S., and White, D. F., *Immunology*, **22**, 219-226 (1972).
- Graf, W. M., and Uhr, J. W., *J. exp. Med.*, **130**, 1175-1186 (1969).
- Möller, G., *J. exp. Med.*, **127**, 291-306 (1968).
- Möller, G., *Transplantation*, **2**, 405-415 (1964).
- Haughton, G., and Cooper, M. Y., *Cell. Immun.*, **8**, 384-394 (1973).
- Abrahams, S., Phillipsad, R. A., and Miller, R. G., *J. exp. Med.*, **137**, 870-892 (1973).
- Brambell, F. W. R., in *Transmission of passive immunity from mother to young*, **18**, 13 (North-Holland Research Monographs, Frontiers of Biology, 1971).
- Jerne, N. K., Nordin, A. A., and Henry, C., in *Cell bound antibodies* (edit. by Amos, B., and Koprowski, H.) 109 (Wistar Institute Press, Philadelphia, 1963).
- Plescia, O. J., and Braun, W., *Adv. Immun.*, **6**, 231-252 (1967).
- Sweet, G. H., and Welborn, F. L., *J. Immun.*, **106**, 1407-1410 (1971).

Activation of suppressor T cells by tumour cells and specific antibody

IMMUNOSUPPRESSION by passively administered antibody has long been known (see ref. 1 for review) and as antibody can cause enhancement of tumour growth^{2,3}, particular attention has been paid to the role of antibody-mediated immunosuppression in tumour immunology. Hellstrom *et al.* have emphasised that blocking factors, most likely antigen-antibody complexes⁴, suppress cell-mediated immunity and thus play a major part in the progression of tumour growth⁵. Such blocking factors may be an important factor in the induction and/or maintenance of some forms of immunological tolerance⁶⁻⁸. But the mechanism remains obscure.

Some thymus-derived lymphocytes (T cells) may suppress

the immune response of other cells^{9,10}. These suppressor T cells¹¹, like antigen-antibody complexes, may play an important part in the induction of some forms of tolerance^{12,13}, and antigen-antibody complexes may exert their immunosuppressive effects indirectly by preferential activation of suppressor T cells. Since the effective cell-mediated immune response to tumours usually requires the presence of T cells, it is difficult to demonstrate an additional suppressor population of T cells by such manoeuvres as thymus deprivation, which may deplete effector as well as suppressor cells.

Previous studies have shown that hyperimmune isoantibody to leukaemia L-1210 can 'centrally' inhibit the development of spleen cell-mediated immunity in C57BL/6 mice¹⁴. Antibody was also shown to inhibit the development of macrophage-mediated immunity¹⁵. Mice treated with antibody lacked the enlarged vacuolated 'activated' macrophages which are usually observed in these mice 10 d after challenge with tumour. The peritoneal cells of mice pre-treated with antibody before inoculation with L-1210 were small, lacked stainable acid phosphatase granules, and showed no attached or engulfed tumour cells. Moreover, the addition of proved cytophilic antibody to L-1210 failed to restore the capacity of these monocytes to attach L-1210 cells. The monocytes could, however, form rosettes with sheep erythrocytes¹⁵ or EL-4 leukaemia cells¹⁶ in the presence of specific cytophilic antibody. The suppression was long lived, lasting 14–18 d, and requiring specific antigen as well as antibody to be produced.

Since the assay for this macrophage function does not require the presence of T cells, we used it to investigate whether suppressor T cells are involved in passive enhancement of tumour growth. Our results suggest they do, since thymus deprivation completely eliminates the suppressive effects of passive antibody, as judged by the ability of macrophages to bind specific tumour cells in the presence of cytophilic antibody.

C57BL/6 male mice from Jackson Laboratories, Bar Harbor, Maine, were thymectomised when 7 weeks old or sham thymectomised. One week later all mice were given 850 rad of X irradiation from a Siemens stabilipan 250 kV source, at a dose rate of 85 rad min⁻¹. On the same day 5×10^6 bone marrow cells from 7–8-week-old syngeneic mice were inoculated into the tail vein.

Two experiments were performed; one 5 weeks after irradiation and the other after 13 weeks. The results of the two separate experiments were similar and the pooled results are given in Table 1. A single dose of 0.4 ml of hyperimmune antibody raised in C57BL/6 mice by multiple immunisations with 5×10^6 L-1210 cells was given intraperitoneally (i.p.) 1 d before tumour. Mice were then challenged i.p. with either 5×10^6 live L-1210 cells or 25×10^6 or 50×10^6 L-1210 cells, killed by previous treatment with mitomycin C (2 mg per 80×10^6 tumour cells at 37° C for 60 min).

Cytophilic antibody (0.3 ml) was injected with 2.5×10^6 L-1210 cells i.p. into mice 10 d later, 5 h before killing.

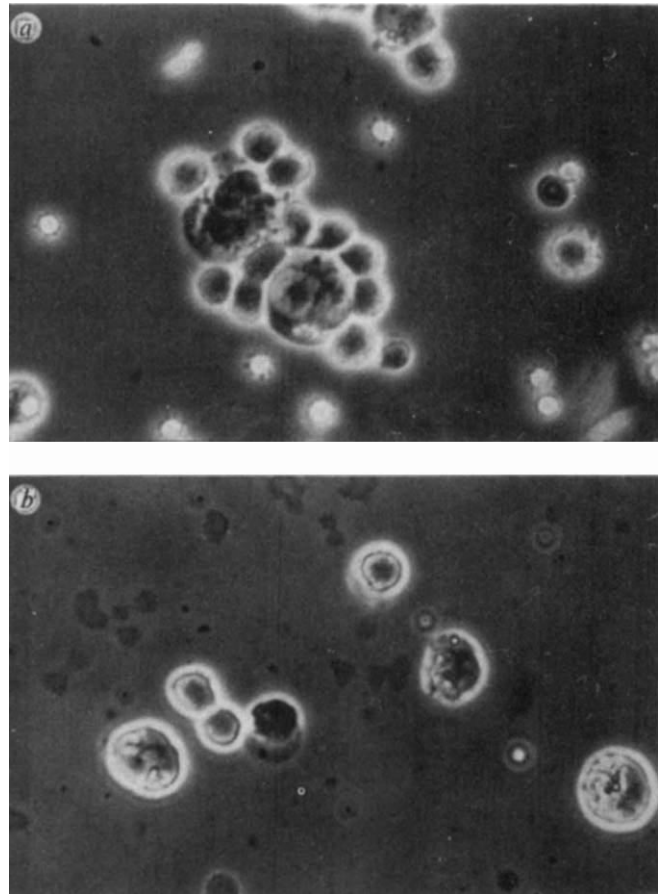


Fig. 1 *a*, Representative field from a 3+ positive slide. Thirteen macrophage cells surround three tumour cells in a clump. *b*, Representative field from a negative slide. Three macrophages without any attached tumour cells are present. Three tumour cells are also present. The dark cell in the middle is unidentifiable.

After killing the peritoneal cavity was lavaged with 6 ml of Fischer's medium. The lavage fluid was centrifuged at 1,000 r.p.m. for 6 min and the cell pellet was gently re-suspended. Covered wet-droplet preparations on a glass slide were coded and then examined 'blind' using a phase contrast microscope. Previous studies have shown that the assay can be done entirely *in vitro* and gives similar results. Results were given an arbitrary 'score' of 0 to 4+ after recording the percentage of macrophages with attached tumour cells and the number of tumour cells that attached to each macrophage. The appearance of the tumour cells and the macrophages (size, presence of refractile granules,

Table 1 Ability of macrophages of lethally-irradiated bone marrow restored C57BL/6 mice given various treatments to attach L-1210 tumour cells in the presence of cytophilic antibody

Treatment		No. of mice	Score assay for rosettes ($\pm$ s.d.)†	% of macrophages with attached tumour cells ($\pm$ 5%)
Thymectomy	Antibody (day 11) Tumour (day 10)			
Sham	—	6	2.3 ± 0.5	50
Sham	+	10	0.4 ± 0.5	< 10
Sham	— (M)*	6	0.0 ± 0	< 10
+	—	7	2.6 ± 0.5	45
+	+	9	2.2 ± 0.4	45
+	— (M)*	4	2.3 ± 0.3	40

* Mitomycin C-treated tumour

† See text

degree of vacuolation and the presence or absence of clumping were also recorded. A score of 0=less than 10% of peritoneal macrophages with attached tumour cells; 1+ = 10–30%; 2+ = 30–50%; 3+ = 50–75%; and 4+ = 75–100% with attached tumour cells.

At least two slide preparations were read for each experimental animal. The mean and standard deviation of the arbitrary scores were calculated for ease of comparison.

Pretreating mice with isoantibody without also giving tumour has no effect on the attachment of macrophages to tumour cells¹⁵. Also, the peritoneal macrophages of normal mice not given isoantibody and inoculated with tumour 10 d previously, bind the tumour extensively whether or not additional cytophilic antibody is given¹⁵. These controls were not repeated in the present studies.

The results (Table 1) show that more than 30% of the macrophages from sham-thymectomised, lethally-irradiated, bone-marrow-reconstituted mice which were otherwise untreated attached L-1210 cells (about 3–5 cells per macrophage) (Fig. 1a) in the presence of cytophilic antibody. If these mice were treated with viable or mitomycin C-killed tumour cells plus isoantibody 10 d before assay, less than 10% of the macrophages attached tumour cells (Fig. 1b). Significantly, pretreatment with isoantibody and L-1210 did not alter the capacity to attach L-1210 cells in the thymus-deprived mice which attached tumour cells in a way indistinguishable from those of the sham-thymectomised untreated ('normal') animals.

These data clearly demonstrate the thymus dependence of the ability of antibody and tumour cells, perhaps acting as complexes, to suppress the binding of tumour cells to macrophages in the presence of cytophilic antibody.

The mechanism by which the T cells exerted their suppressive effect on macrophages is not clear. Others have shown that lymphocytes can make factors which specifically arm macrophages¹⁷. Perhaps they can also make specific factors which act to disarm macrophages by serving as receptors which transmit an inactivating signal when triggered by antigen. Alternatively, the arming factors in excess may act in an inhibitory fashion. Whatever the mechanism, the results clearly show that at least one aspect of antibody-induced immunosuppression is indirectly mediated through T cells. Another form of T-cell dependent antibody-mediated suppression is that produced by anti-allotype antibody¹⁸. Previous work has shown that T cells can suppress the immune functions of other T cells and B cells^{9–13}. Macrophages must now be added to the list of cells affected by suppressor T-cell activity. The precise significance of inhibition of macrophage-mediated immunity in the enhancement phenomenon remains to be determined.

The way in which antigen and antibody, perhaps acting as complexes, activate the suppressor T cells is also unclear. One possibility is that the antibody changes the molecular configuration of the antigen. It is well known that the immunogenicity of antigen is highly dependent on the physical state of antigen²⁰ and in so doing, perhaps, change the T cell response to it. The demonstration by Chan and Sinclair that removal of the Fc fragment from antibody reduces its immunosuppressive qualities²¹ suggests another possible mechanism. Some T cells may have receptors for the Fc portion of some antibodies and the attachment of antigen-antibody complexes at this site might cause them to exert a suppressor function.

In any case, our results offer a possible bridge between two recent observations: the thymus dependence of tolerance induction to some antigens^{12,13} and the presence of immunosuppressive antigen-antibody complexes in otherwise tolerant mice^{6–8}. The suppressive complexes may act through the agency of suppressor T cells.

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- ¹ Uhr, J. W., and Möller, G., *Adv. Immun.*, **8**, 81 (1968).
- ² Kaliss, N., and Molumut, N., *Cancer Res.*, **12**, 110 (1952).
- ³ Kaliss, N., and Kandutsch, A. A., *Proc. Soc. exp. Biol. Med.*, **91**, 118 (1956).
- ⁴ Sjögren, H. O., Hellstrom, I., Bansal, S. C., and Hellstrom, K. E., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 1372 (1971).
- ⁵ Hellstrom, K. E., and Hellstrom, I., *A. Rev. Microbiol.*, **24**, 373 (1970).
- ⁶ Hellstrom, I., Hellstrom, K. E., and Allison, A. C., *Nature*, **230**, 49 (1971).
- ⁷ Phillips, M., Martin, W. J., Shore, A. R., and Wegmann, T. G., *Nature*, **234**, 146 (1971).
- ⁸ Voisin, G. A., *Progr. Allergy*, **15**, 328 (1971).
- ⁹ Katz, D. H., and Benacerraf, B., *Adv. Immun.*, **15**, 1 (1972).
- ¹⁰ Gershon, R. K., *Contemporary Topics in Immunobiology* (in the press).
- ¹¹ Gershon, R. K., Cohen, P., Hencin, R., and Liebhafner, S. A., *J. Immun.*, **108**, 586 (1972).
- ¹² Gershon, R. K., and Kondo, K., *Immunology*, **18**, 723 (1970).
- ¹³ Gershon, R. K., and Kondo, K., *Immunology*, **21**, 903 (1971).
- ¹⁴ Mitchell, M. S., *Cancer Res.*, **32**, 825 (1972).
- ¹⁵ Mitchell, M. S., and Mokyr, M. B., *Cancer Res.*, **32**, 832 (1972).
- ¹⁶ Mitchell, M. S., Mokyr, M. B., and Goldwater, D. J., *Proc. Am. Assoc. Cancer Res. (Abstr.)*, **13**, 62 (1972).
- ¹⁷ Evans, R., Grant, C. K., Cox, H., Steele, A., and Alexander, P., *J. exp. Med.*, **136**, 1318 (1972).
- ¹⁸ Herzenberg, L. A., and Herzenberg, L. A., *Contemporary Topics in Immunobiology* (in the press).
- ¹⁹ Dresser, D. W., *Immunology*, **6**, 378 (1962).
- ²⁰ Conway-Jacobs, A., Schechter, B., and Sela, M., *Biochemistry*, **9**, 4870 (1970).
- ²¹ Chan, P. L., Sinclair, N. R. StC., *Immunology*, **24**, 289 (1973).

Polyanions and lipopolysaccharide acts on different subpopulations of B cells

WITH respect to maturation stage, function, and cell surface constituents, lymphocytes bearing immunoglobulin on their (B cells) comprise a heterogeneous population. It has been suggested¹ that binding of mitogens to membrane surface constituents triggers the events leading to stimulation of cell mitosis. The various B cell mitogens can be divided into different chemical classes and different types of mitogens presumably react with different membrane surface constituents. According to these assumptions we tested whether various B cell mitogens act on the same or on different subpopulations of B cells.

Two kinds of experiments were performed. First, spleen cells of nude mice (BALB/c nu/nu lacking functioning T cells) were cultured with optimal doses of various B cell mitogens or with a combination of two different mitogens (both added to the cultures in optimal doses). Second, BALB/c mice 8–10 weeks old were thymectomised. One week later the mice were lethally irradiated and injected with syngeneic bone marrow cells (TXBM-mice) as described earlier². Spleen cells derived from TXBM-mice were cultured at various times after irradiation and after injection of bone marrow cells (XBM-procedure) with dextran sulphate (DS), with lipopolysaccharide from *Escherichia coli* O 55: B5 (LPS, Difco) or with phytohaemagglutinin-P (PHA, Difco). The results and the experimental details are given in Tables 1 and 2.

As already reported^{3,4} polyanions, for example DS (molecular weight 5×10^5 , Serva, Heidelberg) and double-stranded

Table 1 Effect of combinations of DS, poly (I), poly (C) and LPS on activation of DNA synthesis in nude spleen cells

Mitogen	Dose (μgml^{-1})	c.p.m. per culture	Stimulation index
—	0	852	1.0
DS	50	14.850	18.0
LPS	50	10.640	12.5
poly (I), poly (C)	200	7.071	8.3
DS + LPS	50 + 50	31.950	37.5
DS + poly (I), poly (C)	50 + 200	9.031	10.6
poly (I), poly (C) + LPS	200 + 50	17.380	20.4

Spleen cells suspensions ($2 \times 10^6 \text{ ml}^{-1}$) in a total volume of 3 ml were cultured in RPMI-1640 medium (Biocult) supplemented with 5% fresh heat inactivated (56°C for 30 min) human serum; 1-glutamine (2 mmol ml^{-1}), penicillin (100 U ml^{-1}) and streptomycin ($100 \mu\text{g ml}^{-1}$). The cultures were incubated in a humidified atmosphere of CO_2 : air, 5 : 95 at 37°C for 48 h. The mitogens were dissolved in sterile saline (LPS boiled for 1 h) and 0.1 ml of each solution added to the cultures. Controls received saline instead of mitogens. Two μCi of tritiated thymidine (specific activity 2 Ci mmol^{-1} , Radiochemical Centre, Amersham) were added for the last 4 h of incubation. The radioactivity in the trichloroacetic acid-insoluble fraction was determined as described earlier⁸. The results are expressed as c.p.m. per culture and as stimulation indices (c.p.m. in mitogen treated culture/c.p.m. in control culture). Each value represents the mean of c.p.m. detected in five individual cultures. Standard deviation was less than 10%.

homopolyribonucleic acids are B cell mitogens. As shown in Table 1, spleen cells gave an additive response to DS and LPS or to poly (I) · poly (C) and LPS, when both types of mitogens were added simultaneously to the cultures. These results indicate that LPS and polyanions probably act on different subpopulations of B cells. Since dose response studies (data not given here) showed that higher doses of polyanions gave a smaller response than the optimal doses used in this study, the decreased response of the cells to two different polyanions when they were given simultaneously may indicate that polyanions acts on the same subpopulation of B cells.

One week after the XBM procedure, spleen cells derived from TXBM mice responded to DS and to PHA but not to LPS. LPS-positive cells are first seen in the spleen 2 weeks after XBM-procedure. According to the kinetic studies (Table 2) we postulate that the B cell population responding to DS represents a less matured B cell population than the LPS-reactive B cells. Both populations, however, seem to belong to cells bearing

Table 2 Kinetics of appearance of DS-, LPS- and PHA-reactive cells in spleen of TXBM mice

Time after week XBM-procedure	Stimulation index DS	LPS	PHA
1	6.4	0.7	2.9
2	15.9	2.1	4.7
5	8.4	28.4	5.3
7	10.2	10.1	5.8

Spleen cells derived from TXBM-BALB/c mice (1–7 week after irradiation and bone marrow injection) were cultured for 48 h and the incorporation of ^3H -thymidine into the DNA was measured. Cultures stimulated by PHA received $2 \mu\text{g}$ PHA per ml medium. For details see Table 1. The results are expressed as stimulation indices. Each value represents the mean of c.p.m. detected in three individual cultures. Standard deviation was less than 10%.

immunoglobulin on their surface since pretreatment of a normal spleen cell population by polyvalent rabbit anti-mouse- γ -globulin serum and complement (data not given here) destroyed the capacity of the cells to respond to both DS and LPS but not to PHA. On the other hand it has been shown⁹ that DS but not LPS stimulates DNA synthesis in bone marrow cells of mice. Moreover, the stimulation rate of bone marrow cells derived from TXBM mice by DS was significantly enhanced compared with the reactivity of normal bone marrow cells³. These results and the observation that DS stimulates mice fibroblasts *in vitro* (U. Saar and T. D., in preparation) indicate that the mitotic activity of DS is not restricted to lymphoid cells and that DS

may also be useful for studies on cell differentiation in other systems.

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- ¹ Melchers, F., and Andersson, J., *Transplant Rev.*, **14**, 76 (1973).
- ² Diamantstein, T., Wagner, B., Lage-Stehr, J., Beyse, I., Odenwald, M. V., and Schulz, G., *Eur. J. Immun.*, **1**, 302 (1971).
- ³ Diamantstein, T., Vogt, W., Ruhl, H., and Bocher, G., *Eur. J. Immun.*, **3**, 488 (1973).
- ⁴ Ruhl, H., Vogt, W., Bocher, G., and Diamantstein, T., *Immun.*, (in the press).

Brain-associated tumour antigens demonstrated by immunofluorescence

It has been reported that lymphocytes from patients with cancer react with a basic protein of myelin, suggesting that brain shares antigen(s) with malignant tumours¹. The brain also possesses an antigen in common with the thymus^{2–5}. Using a heterologous anti-brain serum we have investigated the presence of brain-associated antigens in tumour cells and in lymphoid cells.

Anti-rat brain serum was produced in rabbits by three subcutaneous weekly injections of 1 ml of brain homogenate, containing 10 mg nitrogen, in an equal volume of complete Freund's adjuvant⁶; the rabbits were bled 7 d after the third immunisation.

The rat tumours studied were: gliomas and schwannomas induced in the offspring of pregnant DA Agouti rats injected intravenously with ethylnitrosourea (10 mg per kg body weight)⁶, spontaneous mammary carcinomas in inbred Agouti rats, a squamous cell carcinoma⁷ in inbred Wistar rats, and Walker carcinoma in outbred Hooded Wistar rats. Unfixed frozen sections, impression films and smears of brain, thymus, bone marrow, tumours and normal non-lymphoid tissues were examined by standard immunofluorescence procedures⁸. Cell suspensions from tumours, thymus, spleen and lymph node were made by gentle mechanical teasing in tissue culture medium 199. Liver and kidney cell suspensions were made by perfusion of the organs with 10 ml of calcium- and magnesium-free Hanks balanced salt solution containing 0.05% collagenase and 0.1% hyaluronidase, followed by incubation of the diced tissue in the same enzyme solution at 37°C for 30 min. Peripheral blood leukocytes, largely (>90%) lymphocytes, were separated from heparinised blood by centrifugation in a Hypaque-Ficoll gradient⁹.

Sandwich immunofluorescence staining of cell suspensions and tissue preparations was carried out by procedures described in detail elsewhere^{8,10,11}. The conjugate was fluorescein isothiocyanate-labelled goat anti-rabbit globulin with a protein concentration of 0.8 g per 100 ml and a fluorescein: protein molar ratio of 4.2. Before use, it was absorbed with human liver powder and rat liver homogenate, so that by itself it gave no staining of the various microscopical preparations.

The unabsorbed anti-rat brain serum stained the membrane and cytoplasm of all the rat tissues examined. After absorptions⁸ with rat erythrocyte membranes, liver and lung

Table 1 Immunofluorescent staining of rat brain, tumours and thymus by anti-rat brain serum

Antiserum absorbed with	Staining of unfixed frozen sections, smears and impression films of					Membrane staining of cell suspensions of			
	Brain	Tumours†	Thymus	Bone	Normal nonlymphoid tissues‡	Tumours†	Thymus	Bone marrow*	Liver or kidney
Erythrocytes, lung, liver and bone marrow*	++	+	++	—	—	100%	100%	<1%	—
+ Brain	—	—	—	—	—	—	—	—	—
+ Thymus	++	+	—	—	—	—	—	—	—
+ Kidney	++	+	++	—	—	100%	100%	<1%	—
+ Mammary carcinoma	++	—	++	—	—	—	100%	<1%	—
+ Glioma	++	—	++	—	—	—	100%	<1%	—

* Bone marrow cells from thymectomised, irradiated and bone marrow-reconstituted rats.

† Rat gliomas, schwannomas, mammary carcinomas, squamous cell carcinoma and Walker carcinoma.

‡ Normal rat heart, muscle, liver, kidney, testis and skin.

homogenates, bone marrow cells from thymectomised irradiated and bone marrow-reconstituted rats, and dilution 1:4, this antiserum stained both the white matter and the cytoplasm of neurones and glia in unfixed frozen sections and impression films of the brain. Neuronal cytoplasm staining was best seen in sections of the cerebellum where there was strong reaction with the granular layer. The antiserum also stained the surface membranes of 100% of thymocytes, 61% of peripheral blood lymphocytes, 32% of spleen cells, 12% of lymph node cells, 12% of normal bone marrow cells and less than 1% of bone marrow cells from thymectomised, irradiated and bone marrow-reconstituted rats. It also stained the cytoplasm of thymocytes in unfixed frozen sections, smears and impression films, but did not stain smears of bone marrow cells. In contrast, the antiserum stained neither the membranes of cell suspensions of liver and kidney, nor unfixed frozen sections of the following normal rat tissues: heart, muscle, liver,

homogenates of mammary carcinoma, glioma or kidney. Tumour cell membrane staining was inhibited by absorption of the antiserum with brain homogenate and cell suspensions of thymus and tumours (glioma or mammary carcinoma) but not by absorption with kidney cell suspensions. Tumour cell cytoplasm staining was inhibited by absorption with homogenates of brain or tumours (glioma or mammary carcinoma) but not by absorption with homogenates of thymus or kidney (Table 1).

The prevention of brain staining only by brain homogenates points to the presence of an organ-specific brain antigen. The staining of the membranes of thymocytes and thymus-derived lymphoid cells and the inhibition of thymocyte membrane staining by brain or thymic absorptions support the findings of others of "brain-associated thymus antigen". Our observations suggest that there is also a cytoplasmic thymic antigen common to brain and thymus. In addition there seem to be at least two brain-associated tumour antigens: the first is shared by brain and the cell membranes of tumour cells and thymocytes, because tumour cell membrane staining by our antiserum is inhibited by absorption with brain homogenate or thymocyte suspension; the second is shared by brain and the cytoplasm of the tumour cells investigated since tumour cytoplasm staining was inhibited only by absorption with homogenate of brain or either of the two tumours used.

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- Caspari, E. A., and Field, E. J., *Br. med. J.*, **2**, 613-617 (1971).
- Reif, A. E., and Allen, J. M. V., *J. exp. Med.*, **120**, 413-433 (1964).
- Golub, E. S., *Cell Immun.*, **2**, 353-361 (1971).
- Peter, H. H., Clagett, J., Feldman, J. D., and Weigle, W. O., *J. Immun.*, **110**, 1077-1083 (1973).
- Gyongyossy, M. I. C., and Playfair, J. H. L., *Cell Immun.*, **7**, 118-123 (1973).
- Druckrey, H., Ivanovic, S., and Preussman, R., *Nature*, **210**, 1378-1379 (1966).
- Flannery, G. R., Chalmers, P. J., Rolland, J. M., and Nairn, R. C., *Br. J. Cancer*, **28**, 118 (1973).
- Nairn, R. C., *Fluorescent Protein Tracing* third ed. (Livingstone, Edinburgh, 1969).
- Boyum, A., *Scand. J. clin. Lab. Invest.*, **21**, Suppl. 97 (1968).
- Rolland, J. M., Nairn, R. C., and Davies, D. J., *J. Immun. Methods*, **1**, 83-90 (1971).
- Nairn, R. C., Cusdin, C. H., and McNaughtan, J., *Clin. exp. Immun.*, **8**, 835-838 (1971).

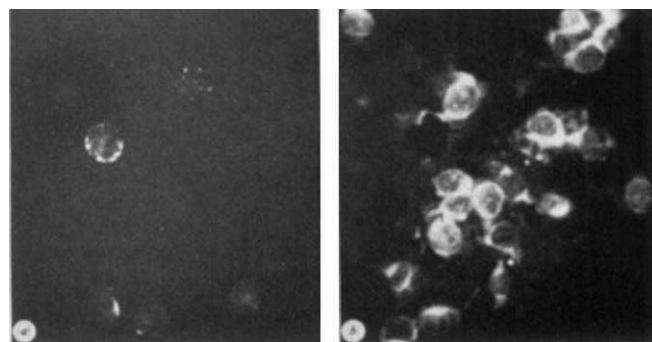


Fig. 1 Immunofluorescent staining of tumour cells by rabbit anti-rat brain serum. *a*, Membrane staining of cell suspensions of rat mammary carcinoma ($\times 268$); *b*, cytoplasm staining of impression films of rat squamous cell carcinoma ($\times 171$).

kidney, testis and skin. Moderate to strong membrane and cytoplasm staining in 100% of cells was, however, observed in all the tumours tested: gliomas, schwannomas, mammary carcinomas, squamous cell carcinoma and Walker carcinoma (Fig. 1). In all cases, no staining was observed in the parallel control tests with preimmune serum, absorbed in the same way as the test serum.

The staining of brain sections was inhibited only by serum absorption with brain homogenates. However, thymocyte membrane staining was inhibited by serum absorption with rat brain homogenate or thymocyte cell suspensions but not by cell suspensions of mammary carcinoma, glioma or kidney, and thymocyte cytoplasmic staining by absorption with homogenates of brain and thymus but not by

Susceptibility of xeroderma pigmentosum cells to chromosome breakage by adenovirus type 12

DNA repair-deficient microbial systems have attracted considerable attention because they offer an insight into the genetic control of sensitivity towards mutagens and carcinogens¹. These studies have become highly relevant to man with Cleaver's discovery² that cells of most patients with xeroderma pigmentosum (XP) have a reduced level of DNA repair synthesis following ultraviolet-irradiation or exposure to several chemical carcinogens³⁻⁶. The impaired repair capacity of XP cells seems to result in their greater sensitivity towards the lethal⁶⁻¹⁰ and chromosome-damaging action¹¹⁻¹² of those physical and chemical carcinogens which induce irreparable DNA alterations. On the other hand the behaviour of XP cells does not differ from that of controls when treated with several potent alkylating mutagens and carcinogens^{5,10,12,13}.

To further characterise the response of DNA repair-deficient cells towards carcinogens and mutagens which belong in different categories, we have examined the effect of infectious and impaired human adenovirus type 12 (AD12) on cultured fibroblasts of three unrelated XP patients and three control subjects. AD12 was used because it readily infects cultured human cells, it replicates within the cell nucleus, it has a capacity to induce chromosome breaks in mammalian cells, including humans cells¹⁴⁻¹⁷, and it forms intranuclear inclusion bodies (IB) which can be used to identify virus-producing cells^{15,16,18,19}. Since the repair capacity of the three XP cultures varied, we examined further whether different degrees of repair deficiency give rise to different sensitivities towards the chromosome-damaging effect of infectious and ultraviolet-impaired AD12.

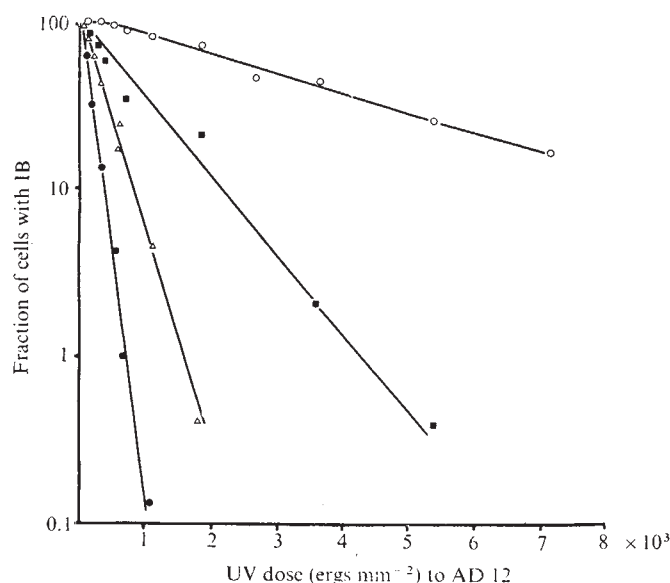


Fig. 1 Effect of ultraviolet irradiation on the capacity of AD12 to form IBs in three XP cell cultures (XP-E, XP-C, XP-K) and control. Samples were taken 48 h after infection. Virus suspensions were spread in a thin layer in a 60 mm Petri dish and irradiated under constant agitation with a Sylvania germicidal lamp (G15T8). At a distance of 7 inch this gave a dose of $30 \text{ ergs mm}^{-2} \text{ s}^{-1}$. Following irradiation the virus preparation was immediately added to the fibroblast cultures. The virus absorption time was 4 h at 37°C . The original nonirradiated AD12 preparation had an input multiplicity of 50 TCD₅₀ per cell. In each sample 2,000 to 2,100 nuclei were screened for the presence of IBs. Two coverslip cultures were used for each sampling point. ○, Controls (average of three nonaffected persons); ●, XP-E; △, XP-C; ■, XP-K.

Cultured fibroblasts were obtained from small skin punch biopsies of three unrelated XP patients and three control persons of comparable age. The stock cultures were kept in 10 cm diameter plates (Falcon plastic) placed in a CO_2 incubator and were fed Eagle's minimum essential medium (MEM) supplemented with 20% foetal calf serum (FCS) and antibiotics. For the experiments monolayers of cells were grown on glass cover slips ($20 \times 20 \text{ mm}$) which were kept in small plastic plates. The level of DNA repair synthesis, which was estimated by measuring the unscheduled uptake of ^3HdR , was about 21% for XP-E, 32% for XP-C and 56% for XP-K as compared to that found in three control subjects following ultraviolet irradiation. The stock preparations of AD12, prototype strain Huie, contained 10^7 TCD_{50} per 0.2 ml as determined by titration in human embryonic kidney cells. The AD12 preparations seemed to be free of PPLO and adeno-associated virus (AAV), as judged by electron-microscopic examination. Cells with replicating virus were identified by the presence of intranuclear inclusion bodies (IB) which are the replication site of adenovirus^{15,16,18,19}. The frequency of IB parallels results obtained on plaque assays^{16,20} and is dependent on the input multiplicity of the virus. IB and chromosome aberrations were analysed on preparations which were pre-treated with citrate solution, fixed with ethanol-acetic acid (3:1) and stained with a 2% solution of aceto-orcein.

The frequency of XP cells which support viral replication was compared with that in control cultures by counting the proportion of fibroblasts containing IB. In samples taken 30, 48, 72 and 96 h after infection with nonirradiated AD12 the frequency of cells containing IB in the three XP cultures examined depended on the ratio of viruses to cells and closely resembled that of controls. But there was a marked difference between XP cells and controls following infection with ultraviolet-irradiated virus. Relatively small doses of ultraviolet-irradiation which did not alter the frequency of IBs in control cultures reduced the capacity of AD12 to replicate and form IBs in the XP cells (Fig. 1). The capacity of ultraviolet-impaired AD12 to form IB was abolished faster in XP cells with a severe repair deficiency than in those with a less affected DNA repair capacity.

There was a slight increase in the frequency of IB-containing XP cells with time after infection with ultraviolet-irradiated (90 to $1,800 \text{ ergs mm}^{-2}$) AD12, but even after 96 h their frequency did not reach the level found in cell populations of nonaffected persons. The extent of recovery of the replicative capacity of ultraviolet-impaired AD12 also depends on the degree of repair deficiency of the host cell. For example, ultraviolet doses of more than $1,800 \text{ ergs mm}^{-2}$ destroyed the capacity of the AD12 to recover its replicative potential in XP-E cells, whereas a slight recovery occurred in the less repair-deficient XP-C and XP-K cells and a restoration of about 60% took place in cells of nonaffected persons.

The extent of AD12-induced chromosome aberration was estimated by counting the number of metaphase plates with at least one chromatid or chromosome break or one chromatid exchange and by calculating the average number of chromosome aberrations per metaphase plate. There was no significant difference between the frequency of XP cells with chromosome aberrations and that of controls when infected with the same numbers of nonirradiated AD12 and sampled 30, 48, 72 or 96 h after infection. But the behaviour of the three XP cell cultures (XP-E, XP-C and XP-K) departed from that of the controls following infection with ultraviolet-irradiated AD12. Virus preparations irradiated with relatively small doses of ultraviolet were less capable of inducing chromatid and chromosome breaks in XP cells during the first two days after infection (Table 1). The frequency of chromosome aberrations was lower in those XP cells which had a severe DNA repair deficiency than in XP cells with a less impaired repair system. This pattern of survival of a viral function, which can be clearly observed in early samples after infection, closely resembles

that of IB formation in XP and normal cells following infection with ultraviolet-irradiated AD12 (Fig. 1).

In the XP cells examined the chromosome damaging action of ultraviolet-irradiated AD12 seemed to become restored at a later stage after infection (Table 1). The severity of chromosome breakage was also greater in samples taken 72 or 96 h after infection. In the 48 h samples chromatid breaks (Fig. 2a) were the major type of aberration, whereas fragmentation of the entire chromosome complement (Fig. 2b), which leads to the formation of micronuclei (Fig. 2c), predominated in the later samples. The IB-forming capacity of AD12 which received a dose of 3,600 ergs mm⁻² was either not restored (XP-E and XP-C cells) or showed only a slight recovery (XP-K cells) within the 96 h period.

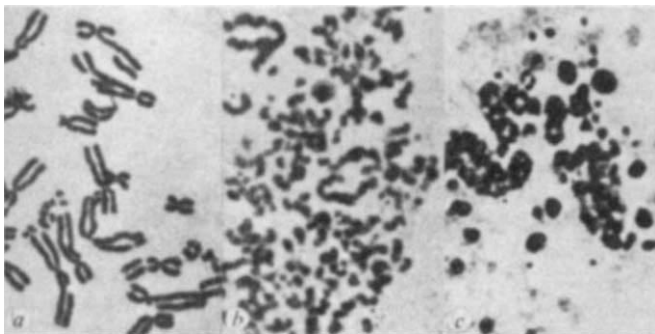


Fig. 2 Part of a metaphase plate of an XP-E culture infected with ultraviolet-irradiated AD12 (720 ergs mm⁻²). a, Sampled 30 h after infection. Several chromatid and chromosome breaks. b, Sampled 96 h after infection. Fragmented chromosome complement. c, Part of a cell with multiple micronuclei of an XP-E culture infected with ultraviolet-irradiated AD12 (720 ergs mm⁻²) and sampled 96 h after infection.

Controls and XP cells seemed to differ in the extent of chromosome damage rather than in the type of chromosome aberrations which followed infection with nonirradiated or ultraviolet-irradiated AD12. Chromosome fragmentation occurs in the examined XP cells as well as in controls (Table 1). Furthermore the nonrandom distribution of chromosome aberrations which was observed in cultured fibroblasts of normal persons¹⁵ and patients with Edward's syndrome¹⁷ also occurred in XP cells following infection with nonirradiated or ultraviolet-irradiated AD12. For example following infection with ultraviolet-irradiated (180 ergs mm⁻²) AD12 59%, 67%, 58% and 65% of all chromosome aberrations accumulated in chromosome 1 and 17 of XP-E, XP-C, XP-K and controls respectively, although their combined length was just over 10% of the entire chromosome complement. Furthermore most

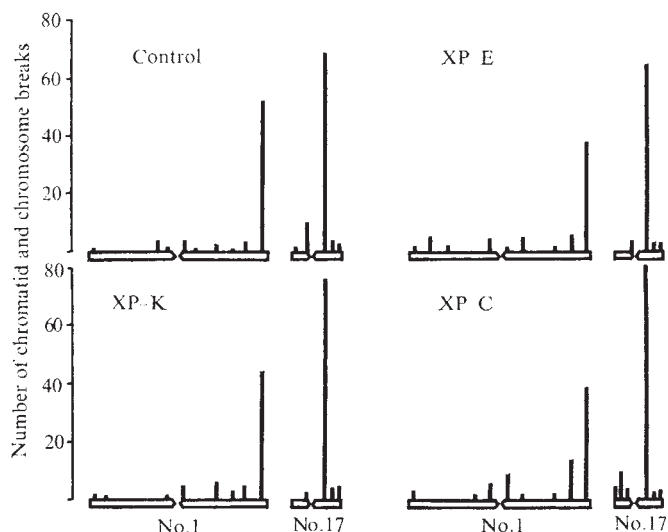


Fig. 3 Distribution of chromatid and chromosome breaks along chromosome 1 and 17 of controls and XP cells (XP-K, XP-E, XP-C) following infection with ultraviolet-irradiated (180 ergs mm⁻²) AD12. Samples were taken 30 h after infection. For each sample, 60 cells with chromosome aberrations were analysed.

chromatid aberrations were located near the end of chromosome 1 and in the region of the secondary constriction of chromosome 17 (Fig. 3), which agrees with previous observations on cells with a normal¹⁵ and trisomic karyotype¹⁷.

Thus the repair of ultraviolet-irradiated viral genomes, and hence the restoration of replication and chromosome-damaging function, occur at lower levels in XP cells than in host cells with an unimpaired DNA repair system. But even in XP cells ultraviolet-irradiated AD12 induces a relatively high frequency of chromosome aberrations, which are manifested because the impaired virus cannot replicate and thus does not cause cell lysis. The data also show that the chromosome-damaging function is more readily restored than the replicative capacity of the virus. This may be because the first property requires only a part of a functional genome²¹. Furthermore, the degree of survival of these two viral functions depends on the level of DNA repair capacity of the host cells. These results can be compared with the reduced T antigen formation and cell transformation in XP cells infected with ultraviolet-impaired SV40²² and the diminished virus production following infection with ultraviolet-irradiated herpes simplex virus²³. The marked increase in chromosome damage at the third and fourth day after infection with ultraviolet-irradiated AD12 is probably caused by a delayed recovery of viral function, either due to a slow-working excision repair in XP cells or to an exchange

Table 1 Frequency and type of chromosome aberrations of xeroderma pigmentosum cells and controls following infection with ultraviolet-irradiated (3,600 ergs mm⁻²) AD12

	Sampling time after infection (h)															
	XP-E				XP-C				XP-K				Control			
	30	48	72	96	30	48	72	96	30	48	72	96	30	48	72	96
Metaphase plates with chromosome aberrations (%) [*]	0	4	12	45	0	6	30	59	0	16	60	79	63	44	31	16
Types of aberrations [†]																
Chromatid breaks (1-9) [‡]	0	100	40	0	0	98	28	0	0	50	4	1	96	25	28	26
Chromatid breaks (10-60)	0	0	9	34	0	2	34	8	0	33	11	4	4	52	44	36
Fragmentation (> 60)	0	0	51	66	0	0	66	92	0	17	85	95	0	23	28	38

^{*}Between 120 to 140 metaphase plates were analysed for each sample. The figures include only chromatid and chromosome breaks since chromatid exchanges were only occasionally seen.

[†]Figures show the percentage of the total number of chromosome aberrations.

[‡]Figures indicate the number of chromatid or chromosome breaks per chromosome complement.

type of repair between viral genomes in the host cell.

In conjunction with previous reports, this study provides evidence that the sensitivity of XP cells varies towards different physical, chemical and viral carcinogens and mutagens (see ref. 24 for review). Similarly, cells of patients with Fanconi's anaemia have an elevated sensitivity to some but not all mutagenic agents²⁵. Cells of patients afflicted with XP and those with Fanconi's anaemia differ in their response towards the same carcinogens. Such selective sensitivities of certain human groups will make the assignment of carcinogen and mutagen levels that are safe or tolerable to all human beings a difficult, if not impossible, task.

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- ¹ Smith, K. C., and Hanawalt, P. C., *Molecular Photobiology Inactivation and Recovery*, (Academic Press, New York, 1969).
- ² Cleaver, J. E., *Nature*, **218**, 652 (1968).
- ³ Stich, H. F., and San, R. H. C., *Mutat. Res.*, **10**, 389 (1970).
- ⁴ Setlow, R. B., and Regan, D., *Biochem. biophys. Res. Commun.*, **46**, 1019 (1972).
- ⁵ Stich, H. F., San, R. H. C., Miller, J. A., and Miller, E. C., *Nature*, **238**, 9 (1972).
- ⁶ Stich, H. F., and San, R. H. C., *Proc. Soc. exp. Biol. Med.*, **142**, 155 (1973).
- ⁷ Cleaver, J. E., *Int. J. rad. Biol.*, **18**, 557 (1970).
- ⁸ Goldstein, S., *Proc. Soc. exp. Biol. Med.*, **137**, 730 (1971).
- ⁹ Takeba, H., Furuyama, J., Miki, Y., and Kondo, S., *Mutat. Res.*, **15**, 98 (1962).
- ¹⁰ Stich, H. F., San, R. H. C., and Kawazoe, Y., *Mutat. Res.*, **17**, 127 (1973).
- ¹¹ Parington, J. M., Delhanty, J. D. A., and Baden, H. P., *Ann. hum. Genet.*, **35**, 149 (1973).
- ¹² Stich, H. F., Stich, W., and San, R. H. C., *Proc. Soc. exp. Biol. Med.*, **142**, 1141 (1973).
- ¹³ Cleaver, J. E., *Mutat. Res.*, **12**, 453 (1971).
- ¹⁴ Stich, H. F., *Progr. exp. Tumour Res.*, **18**, 260 (1973).
- ¹⁵ Zur Hausen, H., *J. Virol.*, **1**, 1174 (1967).
- ¹⁶ Stich, H. F., Avila, L. R., and Yohn, D. S., *Expl. Cell Res.*, **53**, 44 (1968).
- ¹⁷ McDougall, J. K., *Nature*, **225**, 456 (1970).
- ¹⁸ Martinez-Palomo, A., Le Buis, J., and Bernard, W., *J. Virol.*, **1**, 817 (1967).
- ¹⁹ Stich, H. F., Kalnins, V. I., McKinnon, E., and Yohn, D. S., *J. ultrastruct. Res.*, **19**, 556 (1967).
- ²⁰ Rainbow, A. J., and Mak, S., *Radiat. Res.*, **50**, 319 (1972).
- ²¹ Gilead, Z., and Ginsberg, H. S., *J. Bact.*, **92**, 1853 (1966).
- ²² Aarason, S. A., and Lytle, C. P., *Nature*, **228**, 358 (1970).
- ²³ Lytle, C. P., Aarason, S. A., and Harvey, E., *Int. J. rad. Biol.*, **22**, 159 (1972).
- ²⁴ Stich, H. F., Kieser, D., Laishes, B. A., and San, R. H. C., *Proc. Can. Cancer Conf.* 1973 (in the press).
- ²⁵ Sasaki, M. S., and Tonomura, A., *Cancer Res.*, **33**, 1829 (1973).

the flies are old^{11,12}. Russell¹³ reported an increase in induced monosomy in mice by examining the progeny of matings with sex-linked markers. But many aneuploids may be lethal and the dosages used by Russell were large enough to cause loss of chromosomes by breakage. Recent advances in the technique of culturing mouse oocytes *in vitro* (ref. 14, modified by E. P. Evans and C. E. Ford) have made it possible to circumvent these difficulties by examining meiotic chromosomes in second metaphase. The following experiment was designed to examine the effect of low doses of whole-body irradiation on chromosome segregation during first meiotic division in the oocytes of young female mice.

(C₃H×ICR/Swiss) F₁ hybrid females, aged 3 and 6 months, were exposed to 10 r. and 30 r. whole body γ irradiation from a ¹³⁷Cs source at a focal distance of 51 cm with a rate of 29 r. min⁻¹. The dosage was kept low to minimise chromosome breakage. During exposure, the mice were held in pie-shaped compartments of a round plexiglass container. Equal numbers of control mice were handled in identical fashion but were not irradiated. The mice and their paired controls were killed and their ovaries removed within one week of exposure to radiation. No gonadotrophic hormones were used to stimulate oocyte maturation or superovulation.

The oocytes were teased out of the ovaries and those containing a germinal vesicle were incubated in foetal calf serum for 18–23 h to obtain cells in second metaphase. They were then transferred into a hypotonic solution of 1% sodium citrate for 25 min and those in which the germinal vesicle had disappeared were prepared for chromosomal analysis. A maximum of five oocytes were carefully placed on a microslide, excessive hypotonic solution was removed and a tiny amount of fixative (3:1 methanol:acetic acid) was dropped on the cells and quickly blown dry under the heat of a 25 W bulb. The chromosomes were stained with Giemsa. The slides were coded, before the cells were scored, by three technicians. The number of cells on each slide was recorded so that all cells or remnants of cells were accounted for during the analysis.

Metaphase II chromosomes were clearly analysable in 1,149 irradiated oocytes and 1,054 controls. All identifiable chromosome breaks occurred in the centromeric region and could have resulted from premature separation of the chromatids or rupture during slide preparation. They were slightly more common among the irradiated cells (Table 1), but a significant increase was observed only after exposure to 30 r. There were no acentric fragments and small chromatid breaks, if present, could not be identified because of the characteristic shape of metaphase II chromosomes.

Six of the irradiated oocytes had an extra chromosome, that is, 21 chromosomes (Fig. 1). All other oocytes on the same slides were found and those with analysable metaphase chromosomes were positively identified as having 20 chromosomes. It is unlikely, therefore, that the extra chromosome in these six oocytes had strayed from other cells. Since metaphase II chromosomes cannot be karyotyped, it is not possible to identify the extra chromosome in these cells. No oocytes with an additional chromosome were found among the controls. The difference between irradiated and control is statistically significant ($P=0.02$, Fisher exact test).

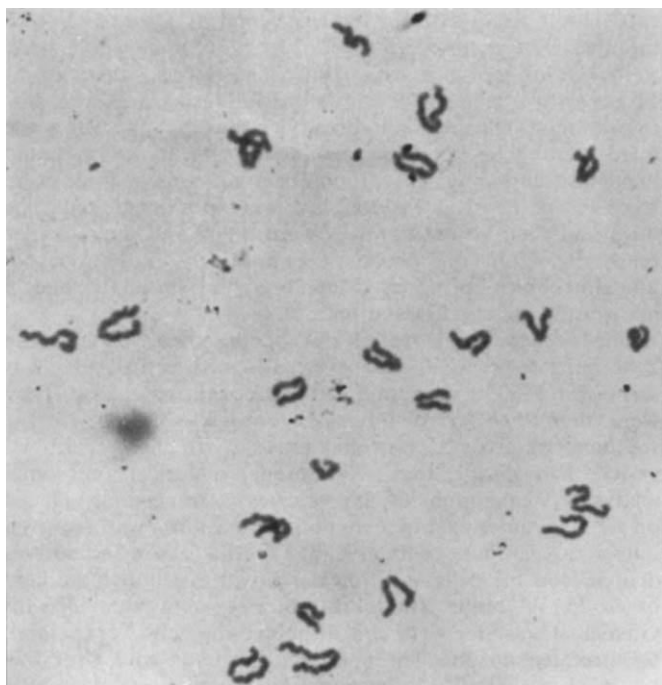
Chromosome loss during slide preparation was most probably the cause of our finding oocytes with less than the haploid number of 20 chromosomes. Cells with fewer than 19 chromosomes have not been tabulated. For each oocyte with 21 chromosomes a complementary cell with 19 chromosomes must have been produced to form the polar body. The reverse should also hold true in the absence of preferential segregation of one type of chromosome complement into the polar body, that is, a similar number of polar body cells with 21 chromosomes could have resulted from the abnormal segregation that produced oocytes with 19 chromo-

Radiation-induced nondisjunction in mouse oocytes

BECAUSE chromosomal trisomy is found with relatively high frequencies in congenital malformations and spontaneous abortions in man, the aetiology of abnormal segregation is a pressing problem. One possible factor is exposure of women to diagnostic X rays^{1–8}. Experiments with *Drosophila melanogaster* indicate that nondisjunction can be induced by exposure of females to X rays^{9,10} especially if

Table 1 Frequencies of numerical and structural aberrations in second metaphase oocytes of mice irradiated *in vivo* compared with nonirradiated controls

Dose (r.)	No. of oocytes analysable	Irradiated				No. cells with breaks	No. of oocytes analysable	Nonirradiated				No. cells with breaks
		19	No. of chromosomes	20	21			19	No. of chromosomes	20	21	
10	426	14	408	2	2	379	11	363	0	5		
20	368	7	355	3	3	421	14	405	0	2		
30	355	13	330	1	11	254	6	246	0	2		
Totals	1,149	34	1,093	6	16	1,054	31	1,014	0	9		

**Fig. 1** Oocytes in metaphase II with 21 chromosomes from mice irradiated *in vivo*.

somes. Since the latter cannot be distinguished from among the total number of oocytes with chromosome loss, the total frequency of nondisjunctional events can be estimated to be approximately double the number of oocytes with 21 chromosomes, that is, 12, or 1%.

The absence of hypermodal cells among more than 1,000 nonirradiated oocytes in our sample agrees with Russell's conclusion¹³ from phenotypic ratios that spontaneous nondisjunction during first meiotic division in the female mouse is exceedingly rare. On the other hand, examination of metaphase II oocytes of a C₃H inbred strain by Röhrborn¹⁵ gave a spontaneous rate of 2.3% with extra chromatids among 128 metaphase II oocytes obtained from females after mating with vasectomised males and not pretreated with hormones. In a relatively limited sample reported by Yamamoto *et al.*¹⁶, no pure trisomy was observed among foetuses of young mice, irradiated and nonirradiated, but both spontaneous and induced nondisjunction was observed among the progeny of aged mice. These conflicting reports probably reflect strain variations as well as differences in experimental design.

Human epidemiological studies have produced contradictory evidence on the effect of radiation on chromosome segregation. But most with negative results show a slight though insignificant increase in nondisjunction. One notable exception was the report on the effect of the atomic bombs in Japan⁷ but cytogenetic investigations are now beginning to reveal a significant increase in sex chromosome aneuploidies among the children of exposed parents¹⁷. The human situation has emphasised the need for experimental

confirmation. The results of our experiment indicate that nondisjunction can be induced in young female mice by exposure *in vivo* to low doses of radiation.

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- ¹ Uchida, I. A., and Curtis, E. J., *Lancet*, ii, 848-850 (1961).
- ² Schull, W. J., and Neel, J. V., *Lancet*, i, 537-538 (1962).
- ³ Sigler, T. T., Lilienfeld, A. M., Cohen, A. M., and Westlake, J. E., *Bull. Johns Hopkins Hosp.*, **117**, 374-399 (1965).
- ⁴ Uchida, I. A., Holunga, R., and Lawler, C., *Lancet*, ii, 1045-1049 (1968).
- ⁵ Marmol, J. G., Scriggins, A. L., and Vollman, R. F., *Am. J. Obstet. Gynec.*, **104**, 533-543 (1969).
- ⁶ Villumsen, A. L., *Environmental factors in congenital malformations* (F.A.D.L.s Forlag, København, Århus, Odense, 1970).
- ⁷ Stevenson, A. C., Mason, R., and Edwards, K. D., *Lancet*, ii, 1335-1337 (1970).
- ⁸ Alberman, E., Polani, P. E., Fraser Roberts, J. A., Spicer, C. C., Elliott, M., and Armstrong, E., *Ann. Hum. Genet.*, **36**, 195-208 (1972).
- ⁹ Mavor, J. W., *J. exp. Zool.*, **39**, 381-432 (1924).
- ¹⁰ Traut, H., *Mutat. Res.*, **1**, 157-162 (1964).
- ¹¹ Patterson, J. T., Brewster, W., and Winchester, A. M., *J. Hered.*, **23**, 325-333 (1932).
- ¹² Uchida, I. A., *Can. J. Genet. Cytol.*, **4**, 402-408 (1962).
- ¹³ Russell, L. B., *Science*, **133**, 1795-1803 (1961).
- ¹⁴ Tarkowski, A. K., *Cytogenetics*, **5**, 394-400 (1966).
- ¹⁵ Röhrborn, G., *Humangenetik*, **16**, 123-125 (1972).
- ¹⁶ Yamamoto, M., Shimada, T., Endo, A., and Watanabe, G., *Nature new Biol.*, **244**, 206-208 (1973).
- ¹⁷ Hamilton, H. D., Awa, A. A., Sofuni, T., Neriishi, S., and Honda, T., *Fourth Int. Conf. Birth Defects Abstracts* (Vienna, 1973).

Very long stretches of free DNA in chromatin

THE arrangement of histones along the DNA in chromosomes has been studied using digestion of the chromatin with DNase¹⁻⁴ and titration of the chromatin with polylysine^{5,6} or certain dyes^{7,8} as probes. The precision of these probes is limited by the extent to which DNA partially covered with histones will react with the enzymes or titration agents used^{7,8}. Furthermore, it has been noted that histones are redistributed along or between DNA fibres under conditions arising during the normal course of isolation and handling of the chromatin⁹⁻¹². This phenomenon may have strongly influenced the results obtained previously.

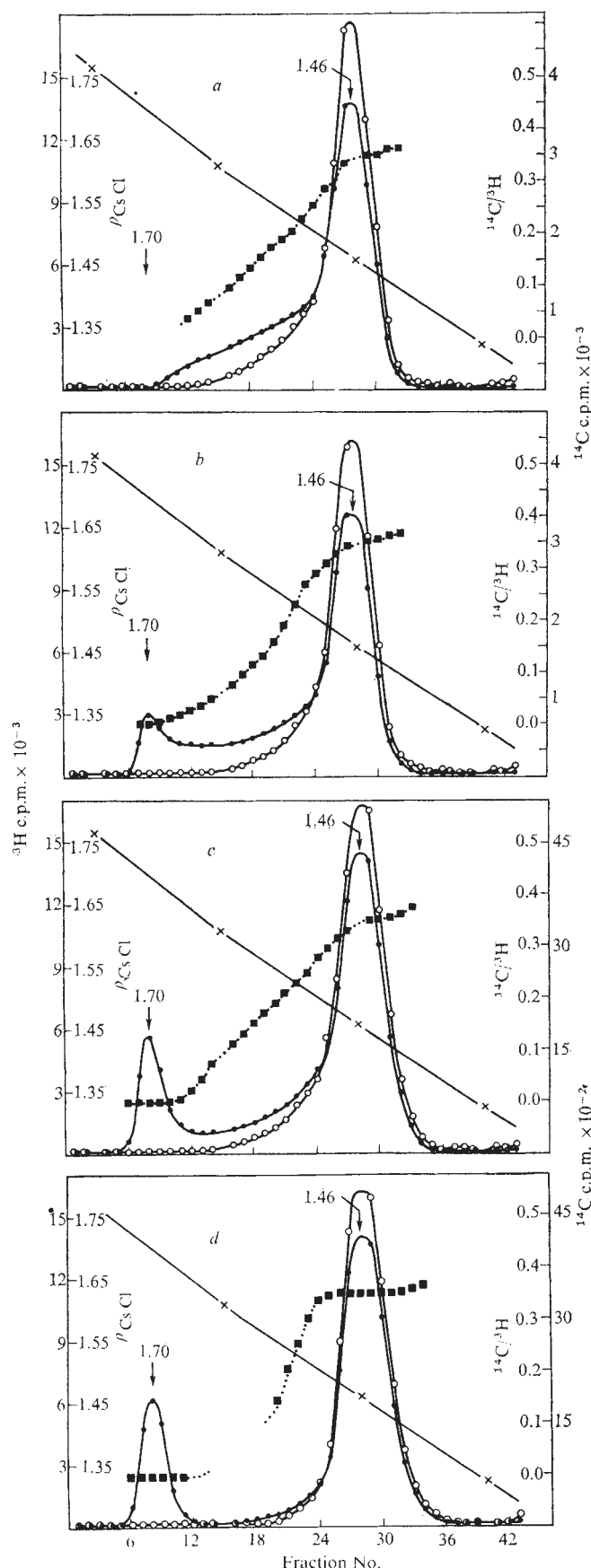


Fig. 1 Long stretches of completely free DNA in DNP-F₁. Mice carrying Ehrlich ascites tumour cells were injected intraperitoneally with a mixture of methyl-³H-thymidine and L-¹⁴C-lysine to obtain a double-labelled chromatin¹⁰⁻¹². Less than 0.1% of the total ¹⁴C counts were in DNA and 0.1–0.2% of the total ³H counts were in protein. The specific radioactivity of the ³H-DNA in the ¹⁴C, ³H-chromatin was usually 2×10^4 – 3×10^4 c.p.m. μg^{-1} . The ¹⁴C/³H ratio was usually about 0.6.

We have tried to examine directly the distribution of histones in the chromatin under conditions that minimise the redistribution of histones¹⁰⁻¹². Our method is based on the direct detection and quantitation of the free DNA segments by equilibrium centrifugation of formaldehyde-fixed deoxyribonucleoproteins (DNP) in CsCl gradients¹¹⁻¹⁵. The use of highly labelled chromatin enables work at low DNP concentrations thus minimising aggregation of the DNP^{11,12}.

Double-labelled chromatin (³H-DNA, ¹⁴C-protein) was used throughout our work. Briefly, unsheared chromatin was prepared from mouse Ehrlich ascites tumour cells which were simultaneously labelled *in vivo* with methyl-³H-thymidine and L-¹⁴C-lysine^{11,12}. The purified chromatin¹⁰⁻¹² was washed and suspended in 1 mM MgCl₂, 1 mM Na₂HPO₄, pH 7.8 buffer to a final concentration of 20–50 $\mu\text{g DNA ml}^{-1}$. Purified total yeast tRNA was added to this suspension to a final concentration of 1 mg ml^{-1} and the mixture was incubated at 0° C for 3–4 h (ref. 10). The swelled gel was solubilised by treatment in a Dounce homogeniser and the resulting soluble ¹⁴C, ³H-DNP lacking histone F₁ and most nonhistone proteins (DNP-F₁) was separated from the tRNA-¹⁴C-protein complexes and from the free tRNA by gel chromatography on Sepharose 2B (refs 10–12). No degradation of histones (as monitored by polyacrylamide gel electrophoresis^{10,11}) could be detected for up to 48–64 h at 0–4° C in either the chromatin gel or the soluble unfixed DNP-F₁.

Soluble ¹⁴C, ³H-labelled DNP-F₁ with an average chain length of about 10 kbases (1 kbases $\equiv$ 1000 bases or base pairs) was fixed with formaldehyde and centrifuged to equilibrium in CsCl (Fig. 1). The majority of the DNP is banded at a density of 1.46 g cm^{-3} with a prominent shoulder at higher densities up to the density of the free DNA (1.70 g cm^{-3}) (Fig. 1a). Thus, even in the sample containing high molecular weight DNA one could observe a significant fraction of material which contained much less protein than the main component.

When the formaldehyde-fixed DNP-F₁ is sheared to an average chain length of about 0.5 kbases (Fig. 1d) the DNP is banded in two homogenous peaks with a negligible amount of material of intermediate density. One peak has the density of free DNA (1.70 g cm^{-3}) and the other has a density of 1.46 g cm^{-3} (Fig. 1d). The first band (1.70) does not contain any ¹⁴C-label (protein). The second band (1.46) is characterised by a nearly constant ¹⁴C/³H ratio throughout the peak area; thus the width of this band is determined almost entirely by diffusion. It should be noted that no protein is removed from the fixed DNP upon shearing in either formaldehyde-free or formaldehyde-containing buffer¹¹⁻¹⁴.

The average length of the stretches of free DNA was estimated from experiments in which the intensity of shearing was varied (Fig. 1). When less intensive shearing was used only a fraction of the free DNA was separated from the DNP-F₁. Under these conditions some of the material is distributed between the densities of the two bands (Fig. 1b and c; compare d). The fraction of the

The ¹⁴C, ³H-DNP-F₁ was obtained as described in the text, then fixed in 1% formalin¹¹⁻¹⁴ and thereafter sheared to the required average chain length by mild or strong sonication or by passage through the water-cooled French press. Appropriate controls have shown that the results depended only on the final average length of DNA obtained but not on the particular method of shearing used. The samples were centrifuged in CsCl at 45,000 r.p.m. for 70–75 h in the SW50.1 rotor (Spinco) at 10° C (refs 11 and 12).

a, DNP-F₁; equilibrium pattern of the unsheared (~10 kbases) preparation in CsCl. 97×10^3 ³H c.p.m.; 28×10^3 ¹⁴C c.p.m.; ~10 μg of DNP. b, As a but after shearing of the fixed DNP-F₁ to an average length of about 4 kbases. c, As a but after shearing to ~1.2 kbases. d, As a but after shearing to about 0.5 kbases. ●, ³H-DNA; ○, ¹⁴C-protein; X, density; ■ ¹⁴C/³H.

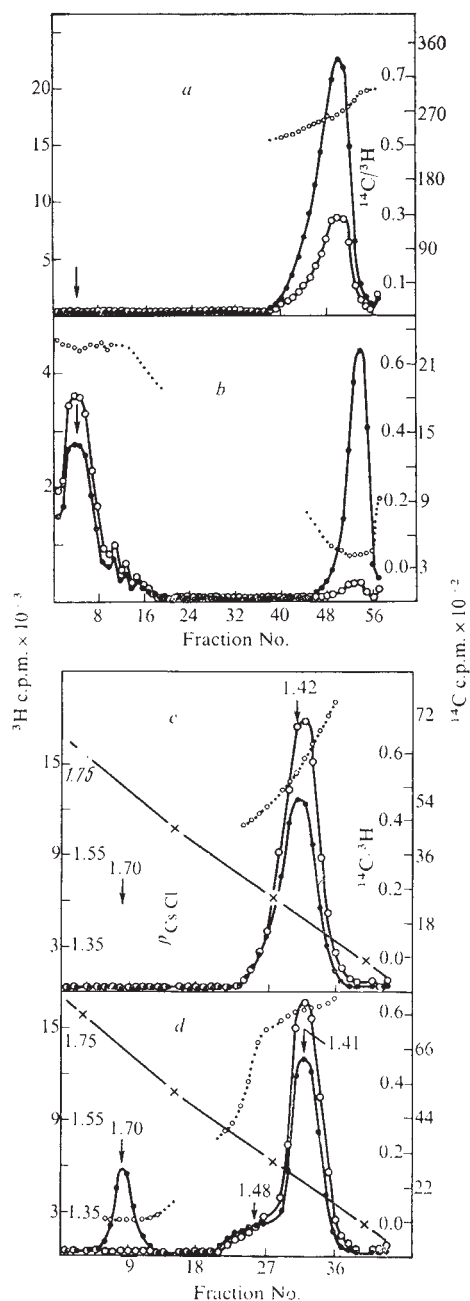


Fig. 2 Free DNA molecules in the sheared chromatin. *a*, Sucrose gradient centrifugation. The ^{14}C , ^3H -chromatin¹⁰⁻¹² was washed in 5 mM TEA-HCl, pH 7.8, then suspended in the same buffer to a final DNP concentration of 10–20 $\mu\text{g DNA ml}^{-1}$ and thereafter sheared to an average DNA length of about 0.5 kbase by a double passage through the water-cooled French press¹⁰⁻¹². The sheared sample was made 1% in formalin and thereafter was centrifuged through a linear 5–40% (w/v) sucrose gradient in the same buffer (35,000 r.p.m.; 3.5 h at 5° C in the SW40 rotor). *b*, As *a* but before layering on to gradient an equal volume of 0.30 M NaCl, 4 mM MgCl₂, 5 mM TEA-HCl, pH 7.8 was added to the sheared chromatin. After 15 min at 0° C the sample was fixed in 1% formalin and thereafter centrifuged as above. *c*, CsCl equilibrium pattern of the sheared chromatin fixed in 5 mM TEA-HCl, pH 7.8 (see *a*). *d*, CsCl equilibrium pattern of the sheared chromatin fixed in 0.15 M NaCl, 2 mM MgCl₂, 5 mM TEA-HCl, pH 7.8 (see *b*). ●, ^3H -DNA; ○, ^{14}C -protein; ■, $^{14}\text{C}/^3\text{H}$; X, density. Arrows in *a* and *b* indicate the position of a dense sucrose shelf.

To achieve a 90% recovery of the DNP in *d*, CsCl centrifugation was carried out in the presence of 0.5% Sarcosyl NL97. Free DNA was recovered quantitatively either with or without Sarcosyl (data not shown).

material of intermediate density consists of DNA-histone complexes linked to remaining stretches of the free DNA. This material is almost undetectable when shearing is more intensive (Fig. 1*b–d*). The lengths of DNA molecules isolated from CsCl gradients were determined by sucrose gradient centrifugation in the presence of internal sedimentation markers (data not shown). One can estimate that the number average length of stretches of free DNA in the DNP-FI is ~ 4 kbases. The variability in length of free DNA segments in the DNP-FI remains unknown. It seems, however, that there are few, if any, segments of free DNA longer than ~ 10 kbases (since no free DNA is present in the unsheared DNP-FI preparation; Fig. 1*a*) and there are almost no segments of free DNA shorter than 1 kbase (since shearing to ~ 0.5 kbase results in an almost complete separation of free DNA segments; Fig. 1*d*). These estimates would indicate the maximal and minimal values while the actual variability of the chain length of free DNA in the DNP-FI may be, of course, much lower.

The average length of histone-covered DNA in the DNP-FI is about four times that of the free DNA (since free DNA constitutes 20–22% of the total DNA; Fig. 1*d* and thus equals about 14–16 kbases. It may be estimated that a stretch of 16 kbases of DNA covered with four histone fractions to a protein/DNA ratio of 1.1 (ref. 14) would be occupied by about 8×10^2 histone molecules (assuming an average molecular weight of 13,500 for the histone). These four histone fractions are nearly uniformly distributed within the histone-covered stretch of DNA, as the fragmentation of DNA to ~ 0.5 kbase (corresponding to ~ 25 bound histone molecules) reveals no heterogeneity in histone distribution within histone-covered 'blocks' (Fig. 1*d*).

It was shown previously, that if the unfolding of chromosomal DNP is carried out in the absence of Mg²⁺ ions all DNA-bound histones may be redistributed¹². This particular type of redistribution is completely blocked in already unfolded DNP preparations. If the unfolding-induced dedistribution of histones is allowed, it leads to a considerable decrease of the average length of free DNA segments in the unfolded, histone FI-depleted DNP¹².

The implications of the above findings will be discussed below (see Fig. 5).

Chromatin which was hydrodynamically sheared in a low (0.005) ionic strength buffer lacking divalent cations, sediments in a sucrose gradient as a single peak with a significant internal heterogeneity (Fig. 2*a*). The same DNP, when fixed with formaldehyde and centrifuged to equilibrium in a CsCl gradient, is banded as a single peak with even higher internal heterogeneity (Fig. 2*c*).

The main result of this part of the work is that addition of approximately physiological concentrations of NaCl and/or MgCl₂ to the sheared, unfixed chromatin preparation results in the appearance of a bimodal distribution of the DNP after centrifugation in either sucrose or CsCl gradient (Fig. 2). For example, after a shift to 0.15 M NaCl, 2 mM MgCl₂, 5 mM triethanolamine (TEA)-HCl, pH 7.8, a significant (18–19%) proportion of completely free DNA molecules appears in the sheared chromatin (Figs 2*b, d* and 3). The average length of either total or free DNA in the experiment presented in Fig. 2 equals about 0.5 kbase. If the shearing of chromatin is less intensive, producing DNA chains with average length of 2–3 kbases, free DNA molecules are still present in the sheared chromatin after addition of NaCl and/or MgCl₂ but their percentage is decreased to 2–3% of the total DNA (data not shown).

An additional feature of the sedimentation pattern of the sheared chromatin in 0.15 M NaCl, 2 mM MgCl₂ (Fig. 2*b*) is that DNP particles, after liberation of the free DNA, sediment 20–30 times faster than the sheared chromatin before addition of NaCl and/or MgCl₂ (Fig. 2*b*; compare Fig. 2*a*). Electron microscopy has shown that this effect is

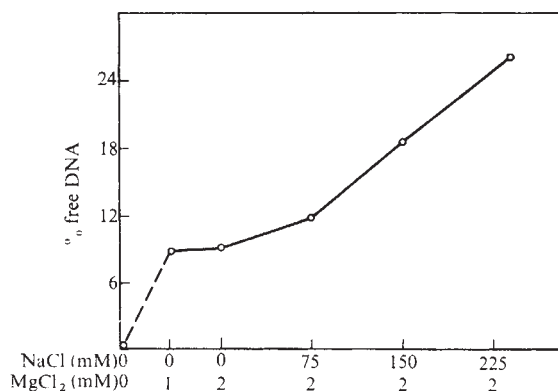


Fig. 3 Percentage of free DNA molecules in the sheared chromatin as a function of the ionic strength of solution. Each point was obtained by calculating the percentage of the free DNA in a CsCl equilibrium pattern of the sheared chromatin (Fig. 2c and d) which was preincubated in 5 mM TEA-HCl, pH 7.8 plus stated concentrations of NaCl and MgCl₂.

due to a collapse of the DNP accompanied by some aggregation of the DNP (unpublished data).

Percentage of free DNA molecules obtained from sheared chromatin is increased from zero in 5 mM TEA-HCl, pH 7.8 to 26% in 0.24 M NaCl, 2 mM MgCl₂, 5 mM TEA-HCl, pH 7.8 (Fig. 3). At sufficiently high (0.10–0.25 M) NaCl concentrations Mg²⁺ is not necessary for the appearance of free DNA; the same result could be obtained also with NaCl/Na-EDTA mixtures.

After a shift to 0.15 M NaCl, 2 mM MgCl₂ a small fraction (5–7%) of the DNP was reproducibly found cosedimenting with free DNA molecules in the upper part of the sucrose gradient (Fig. 2b). This slowly sedimenting DNP banded in CsCl at 1.48–1.50 g cm⁻³ and could thereby be separated from the free DNA (Fig. 2d and unpublished data). The significance and origin of this material remains unclear.

Ehrlich ascites tumour cells labelled in the usual manner (see above) were suspended in 0.15 M NaCl, 2 mM MgCl₂, 5 mM TEA-HCl, pH 7.8 and passed once through a water-cooled French press under a pressure drop of about 1.5 kbar. The preparation was fixed in 1% formalin within a few seconds of shearing and thereafter centrifuged to equilibrium in CsCl gradient (Fig. 4). About 10–12% of the total DNA obtained is banded in the region of free DNA (Fig. 4). Appropriate controls (unpublished) have shown

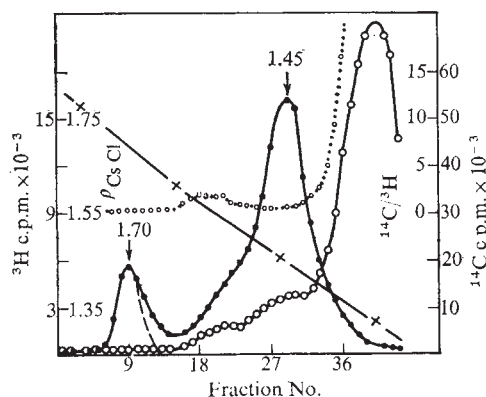


Fig. 4 Free DNA molecules in homogenate of whole cells. *a*, An aliquot of the ¹⁴C, ³H-labelled ascitic fluid was immediately diluted ~200 times into 0.15 M NaCl, 2 mM MgCl₂, 5 mM TEA-HCl, pH 7.8, then sheared to an average DNA length of about 0.5 kbase by a single passage through a water-cooled French press and thereafter fixed in 1% formalin within a few seconds after shearing. The sample was centrifuged to equilibrium in CsCl as described in the legend for Fig. 1. ●, ³H-DNA; ○, ¹⁴C-protein; ○●, ¹⁴C/³H; X, density.

that most if not all free DNA is of nuclear origin. A reproducible, but yet unexplained feature of the CsCl pattern of the whole cell homogenate is the relatively high density of the main DNP peak (1.45 g cm⁻³ compared with 1.41–1.42 g cm⁻³ for purified sheared chromatin; Fig. 2c and d). These data indicate that in homogenates of whole cells under physiological ionic conditions long molecules of the free DNA are present.

At the present time it is unclear whether the very long stretches of completely free DNA discovered in this work have a functional significance with regard to transcription or whether they are structural features of the chromosome irrelevant to transcription. Studies on the renaturation of the free DNA and hybridisation of pre-mRNA to free DNA are in progress and should clarify this question.

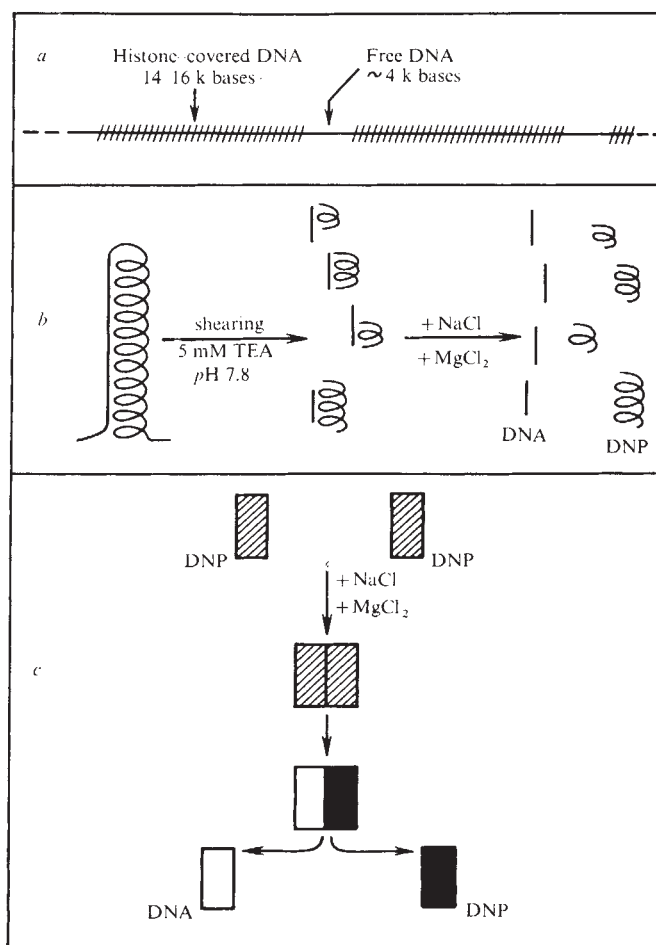


Fig. 5 Possible mechanisms of formation of free DNA molecules in the sheared chromatin. *a*, An experimentally observed mode of arrangement of histones other than FI along unfolded chromosomal DNA (see Fig. 1 and the text). *b*, Generation of free DNA molecules in the sheared chromatin according to an 'asymmetric hairpin' model of DNA packing in a chromomere (see the text). *c*, Alternative model for the generation of free DNA molecules from sheared chromatin. Each DNP particle contains only one DNA molecule (see text for details).

At present we would like to consider another question: if there are only DNP particles in the sheared, soluble chromatin and no free DNA molecules (in the absence of NaCl and/or MgCl₂; see Fig. 2) what is the mechanism of generation of free DNA molecules on shifting to physiological ionic conditions? There are two explanations (Fig. 5b and c). According to the first model shearing of chromatin in a low (~0.005) ionic strength buffer lacking divalent cation produces DNP particles which contain two

(or more) noncovalently linked DNA molecules per particle. Such DNP particles are interpreted as fragments of the 'asymmetric hairpin' (Fig. 5b). One branch of the hairpin is thought to contain tightly supercoiled DNP whereas the other branch is in a relatively extended form. Addition of 1–2 mM MgCl₂ and/or 0.1–0.2 M NaCl induces separation of the branches, the final result being the liberation of free DNA molecules (which correspond to the extended branch) and of the supercoiled DNP particles (Fig. 5b). The asymmetric hairpin model of DNA packing in the chromatin was suggested to explain the observed distribution of histones other than F1 along unfolded chromosomal DNA (Fig. 5a) and also to explain a particular type of histone redistribution which occurs only at the moment of DNA unfolding in the chromatin but not after it (ref. 12; see also above). Furthermore, it should be noted that the average length of histone-covered plus histone-free DNA stretches (~20 kbases; see Fig. 5a) is comparable with the average length of transcriptional units in higher eukaryotes^{16–17}. Although the asymmetric hairpin model (Fig. 5b) is formally consistent with the present experimental data, an alternative model (Fig. 5c) now seems more likely. According to this model, each DNP particle in the sheared chromatin contains only one DNA molecule. In the presence of 1–2 mM MgCl₂ and/or 0.1–0.2 M NaCl, interactions between different DNP particles, for example, during 'dimer' or aggregate formation (Fig. 5c) induce an 'asymmetric' redistribution of proteins between DNP particles in the dimer or in aggregates of higher order. As a result dissociation of such a complex produces one molecule of free DNA and one or a few molecules of DNA associated with increased amount of protein (Fig. 5c). At present a more detailed model is not possible.

The second model (Fig. 5c) is consistent with all results obtained with sheared chromatin (Figs 2 and 3 and unpublished data) including the dependence of the final percentage of free DNA on the ionic strength of solution (Fig. 3). Work is now in progress to distinguish between the two models (Fig. 5b and 5c).

Although the *in vivo* significance of the long free DNA segments in the chromatin is not yet understood, it is clear that the fine structure of chromatin is strongly dependent on the ionic conditions of the medium. One should be careful when attempting to assess the significance of results of earlier studies on chromatin structure, which were carried out mainly at low ionic strength^{16,17}.

We thank V. V. Bakayev and A. A. Bayev, jun. for collaboration at later stages of the work, Dr V. N. Soyfer for providing ultracentrifuge facilities and N. N. Dobbert for technical assistance.

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- ¹ Murray, K., *J. molec. Biol.*, **39**, 125 (1969).
- ² Clark, R. J., and Felsenfeld, G., *Nature new Biol.*, **229**, 101 (1971); *ibid.*, **240**, 226 (1972).
- ³ Itzhaki, R. F., *Biochem. J.*, **122**, 583 (1971); **125**, 221 (1971).
- ⁴ Marushige, K., and Bonner, J., *Proc. natn. Acad. Sci. U.S.A.*, **68**, 2941 (1971).
- ⁵ Itzhaki, R. F., and Cooper, H. K., *J. molec. Biol.*, **75**, 119 (1973).
- ⁶ Angerer, L. M., and Moudrianakis, E. N., *J. molec. Biol.*, **63**, 505 (1972).
- ⁷ Li, H. J., and Chang, C., *Biochem. biophys. Res. Commun.*, **47**, 883 (1972).
- ⁸ Billing, R. J., and Bonner, J., *Biochim. biophys. Acta*, **281**, 453 (1972).
- ⁹ Jensen, R. H., and Chalkley, R., *Biochemistry*, **7**, 4388 (1968).
- ¹⁰ Ilyin, Yu. V., Varshavsky, A. J., Mickelsaar, U. N., and Georgiev, G. P., *Eur. J. Biochem.*, **22**, 235 (1971).

- ¹¹ Varshavsky, A. J., and Georgiev, G. P., *Biochim. biophys. Acta*, **281**, 669 (1972).
- ¹² Varshavsky, A. J., and Georgiev, G. P., *Molec. Biol. Rep.*, **1**, 143 (1973).
- ¹³ Ilyin, Yu. V., and Georgiev, G. P., *J. molec. Biol.*, **41**, 299 (1969).
- ¹⁴ Ilyin, Yu. V., Varshavsky, A. J., and Georgiev, G. P., *Molec. Biol., U.S.S.R.*, **44**, 821 (1970).
- ¹⁵ Hancock, R., *J. molec. Biol.*, **48**, 357 (1970).
- ¹⁶ Simpson, R. T., *Adv. Enzymol.*, **38**, 41 (1973).
- ¹⁷ Huberman, J. A., *A. Rev. Biochem.*, **42**, 355 (1973).

Erratum

In the article 'Relative-distance Machian theories' by J. B. Barbour (*Nature*, **249**, 328; 1974) paragraph 7, line 5 should read:

of particles, and their derivatives $\dot{r}_{ij} = dr_{ij}/d\lambda$ with respect

Equation (1) should read:

$$L = \Psi \Gamma \quad (1)$$

where

$$\Gamma = (\sum_{i < j} m_i m_j \dot{r}_{ij}^2)^{1/2}, i = 1, \dots, N (\dot{r}_{ij} = dr_{ij}/d\lambda)$$

$$\Psi = \sum_{i < j} m_i m_j / r_{ij}$$

Paragraph 7, penultimate line should read:

pendent of the time parameter (a sum like $(\Psi + \Gamma)d\lambda$ would

Equations (2) and (3) and the intervening explanation should read:

$$L = \Psi [\sum_{i < j} m_i m_j (\dot{x}_i^2 - 2\dot{x}_i \dot{x}_j + \dot{x}_j^2)]^{1/2} \quad (2)$$

and the Euler-Lagrange equations

$$\frac{d}{d\lambda} (\partial L / \partial \dot{x}_i) = \partial L / \partial x_i$$

are

$$\frac{d}{d\lambda} \left\{ \frac{\Psi m_i}{\Gamma} \left[\sum_{j \neq i} m_j (\dot{x}_i - \dot{x}_j) \right] \right\} = \frac{d}{d\lambda} \left\{ \frac{\Psi m_i}{\Gamma} \left[\dot{x}_i (M - m_i) - \sum_{j \neq i} m_j \dot{x}_j \right] \right\} = \frac{\Gamma \partial \Psi}{\partial x_i}$$

We can now specialise both λ ; taking

$$d\lambda = ds = (\sum_{i < j} m_i m_j \dot{r}_{ij}^2)^{1/2}, \text{ i.e. } \Gamma = 1,$$

and the coordinate system, taking it such that

$$\sum m_i \ddot{x}_i = 0 \text{ (here } d\lambda = ds)$$

In these special frames, which are distinguished by the uniquely determined relative motion, the equations of motion simplify to

$$M \frac{d}{ds} (\Psi m_i \dot{x}_i) = \frac{\partial \Psi}{\partial x_i} \quad (3)$$

In the last line, for ψ read Ψ

Equation (4) and the next line should read:

$$m_i d\dot{x}_i/dt = (1/M\Psi) \partial \Psi / \partial x_i \quad (4)$$

where $\gamma = 1/M\Psi$ is a gravitational constant that is determined.

Page 329, paragraph 5, line 2 should read:

linear in the $\dot{r}_{ij}$ s to L (gyroscopic-type forces) or multiplying L

Equation (5) should read

$$m_i d\dot{x}_i/dt = (1/M\Psi) \partial \Psi / \partial x_i + (1/M\Phi) \partial \Phi / \partial x_i \quad (5)$$

reviews

Since India met Eurasia

Ecology and Biogeography in India
Edited by M. S. Mani. (Monographiae
Biologicae, Vol. 23.) Pp. xix+773.
(Junk; The Hague, 1974.) 190 guilders.

INDIA (including Pakistan, Ceylon (now Sri Lanka), Nepal, Sikkim, Bhutan and Burma) provides a particular fascination to biogeographers, for it is the arena where a southern continent, detached from the disintegrating Gondwana continental block in the vicinity of south-east Africa and Madagascar apparently before the Maestrichtian towards the end of the Cretaceous period, finally traversed the Tethys Sea and collided with the southern shores of Eurasia perhaps in the Oligocene. The Indian Peninsula and Ceylon is the surviving fragment, but the Laccadive, Maldive, Chagos and Mascarene archipelagos represent submerged mountain summits of a larger part that collapsed as the great scarp of the western Ghats rose, consequent on the underthrusting of the northern margin of this continental plate beneath the southern margin of Eurasia; the other consequences of this underthrust have been the creation of Ganges-Indus plains and the massive Tertiary orogene that lifted the Himalayas.

It may be supposed that two tropical Cretaceous faunas and floras, having evolved in isolation, met then for the first time: an essentially insular and probably largely oceanic rain forest biome from the south, and a much more diversified continental array in the north among which were nevertheless the once vast but already retreating rain forests which stretched as far as western Europe in the Eocene, whose heirs now clothe the hills of western Malesia and south-east Asia. Subsequently the uplift of the Himalayas has created whole new environments for colonisation and, with the Pleistocene glaciations, has radically altered regional climate, while man has for long and increasingly destroyed or impoverished the Indian environment.

It is the biogeographers' task to explain the distribution of modern biomes in terms of past events, and to trace the origin, migration and diversification of their component taxa. His only direct evidence is palaeontological, which in India is almost completely lacking between the lower Cretaceous and Miocene, and in the Peninsula

decidedly scant thereafter. He must therefore fall back on an unavoidably speculative approach; but, by careful analysis of the phylogeny and ecology of selected groups, whose ecology, behaviour and structure prevent rapid and distant dispersal in relation to their distribution, deductions from repeating patterns can be made. Here he is aided by the rich and distinctive biomes of the Mascarenes, including Madagascar, which contain representatives of many groups that are unable to traverse any but the shortest breaks in their chosen habitat, and which provide valuable comparison with those of the Andaman and Nicobars to the north-east and of northern continental origin.

Professor Mani's massive compendium largely fails to do this: it suffers from inadequate integration of the contributions from different authors, and overreliance on evidence from coincidence of modern distribution patterns alone, presented by lists of species whose ecology, and phylogenetic relationships where they exist, are either not discussed or are overgeneralised. The five introductory chapters on physiography, geology, climate and soils present valuable detail, but lack integration; as one example in many, a section on the microclimate within selected Indian crops is in no way integrated with the theme of the book. The editor's own chapters in particular suffer from lack of maps to summarise the mass of geographical detail and place names quoted. The five chapters on vegetation are particularly thin on ecology and contain many errors of detail. Ceylon is said to contain few endemic genera and species, yet in fact almost one third of the angiosperm species are endemic; *Impatiens* and *Apostasiaceae* are wrongly stated not to occur in Malesia; *Shorea* is described as a characteristic genus of the western Ghats forests, yet the only species in fact occurring, *S. roxburghii*, is extremely local and at the edge of its range there. The absence of tropical Amentiferae—Fagaceae, Juglandaceae, Myricaceae and Hamamelidaceae—and the extreme poverty of Ericaceae and Coniferae is a salient feature of the peninsula and Ceylon demanding phytogeographic discussion, but there is none; the single native peninsular conifer is merely cursorily mentioned as *Podocarpus latifolius* in one

chapter, *Decussocarpus latifolius* in another. Nowhere does the contrasting biogeography and ecology of forest and grassland in the peninsular mountains receive the interpretation it deserves, and indeed these mountains are stated to lack altitudinal zonation which is quite incorrect.

The editor is a zoologist, and the main emphasis is zoological, the five chapters on regional biogeography being in fact entirely zoogeographical. This is reasonable and must depend on availability of authors (though their titles are misleading), but once again the integrated approach is lacking. The book's introduction stimulates the reader with controversial statements dogmatically presented but he is ultimately disappointed to find that, though they are often repeated, their substantiation is never realised: where is the evidence that the original Gondwana flora and fauna were evolutionary "stagnant" or "degenerating" when the Asiatic land connection became established? Indeed, though extensive descriptions of subsequent migrations in and out of the peninsula are included, where in the whole book are the taxa of acclaimed Gondwana origin, upon which this discussion must depend, convincingly identified by reasoned argument? One is led to suspect that, in spite of disclaimers, the evidence lies merely in the evocative but probably irrelevant relict distribution patterns of a wide range of species of clearly different affinities (and origins?) in peninsular biomes that have become fragmented in Pleistocene to recent times.

Yet the evidence is there for the searching. An integrated approach would, for instance, compellingly suggest that the mighty dipterocarp trees, whose present centre of distribution is in the western Malesian rain forests which they so distinctively dominate, are extraordinarily successful immigrants whose origin must be sought in the so-called degenerate and stagnant Gondwana biomes. The distinct distribution of interfamilial links such as the genus *Axinandra*, as well as the isolated and distinctive Nepenthaceae, demand discussion in relation to their ecology. Four chapters are in fact provided on the biogeography of specific animal groups; among them P. K. Sen-Sarma's treatment of the termites, and K. C. Jayaram's of the freshwater fishes, amphibians and reptiles, con-

taining ideal taxa for biogeographical analysis (unlike the butterflies of D. J. Holloway, which are likely to be of too recent origin to have existed in the peninsula before it became disconnected from Africa). Centres of origin should be traceable, but the discussion never reaches a sufficiently detailed level to convincingly do so. Professor Hilary Cruz's work on the ecology of the extraordinary endemic agamid genera in the hill forests of wet zone Ceylon could, for instance, have provided the basis for such a discussion but his work is not mentioned.

The biogeography of the Indian region is not, of course, that of the peninsula alone; separate chapters are devoted to the Indo-Gangetic plain, and the Himalayan region. But though much information is presented as in the rest of the book, these chapters are marred in the same manner. The chapters on the impact of man, and the ecology of 'tribal' man (by P. Lal) are welcome and useful, as are those on recent faunal impoverishment (A. K. Mukherjee), the ecology of vertebrates of the Indian desert (I. Prakash) and the mammals of Assam (G. V. Kurup).

The book is well designed and printed, though spelling errors, particularly in scientific names, are not infrequent.

P. S. ASHTON

Anatomy of experiments

Design of Experiments: A Realistic Approach. By V. L. Anderson and R. A. McLean. Pp. xvii+418. (Statistics: Textbooks and Monographs.) (Dekker: New York, February 1974.) \$19.75.

I HAVE long contemplated the value of a book that would discuss in detail a few experiments in which statistical concepts were vital to the design, the analysis and the interpretation. The declared objective of the book under review is "to express rather complex ideas on how and why scientific investigators should design experiments". Is this the book I want?

Certainly it has many merits. The authors employ a wide range of examples to illustrate the theory and practice of experimental design, choosing these largely (but not entirely) from manufacturing industries of which they obviously have much experience. For the teacher, the examples analysed fully and others outlined as problems for the student will prove useful sources of ideas. Nevertheless, I was disappointed to find attention concentrated on questions that can be answered in terms of currently available theory of linear models for observations. On the structure of analysis of variance and on variance components,

the presentation is good. Little is said about reasons for choosing particular designs. The interpretation of results remains on the statistician's desk, and is not carried to the field of application. Computational methods and problems are not mentioned: even the existence of standard programs and the desirable features of computer input and output are neglected. The anatomy of analysis of variance is thoroughly displayed, but the developmental and operational physiology of experimentation remains unexplained.

The book proceeds through standard designs, from completely randomised to lattices and factorials, in a manner at times refreshingly different from other texts. Without entering deeply into combinatorial problems, clear accounts are given of the partitioning of sums of squares. The dependence of analysis on randomisation and of significance tests on variance components is well emphasised, and the emphasis on the inference space is often illuminating. The text is enlivened by many comments that reflect the authors' familiarity with the particular examples, increasing my regret that they did not choose to make that familiarity a distinctive feature at the expense of some of the more conventional algebra. Had they done so, they might have let the reader see the severe limitations of the Newman-Keuls and other multiple comparison tests which so seldom represent any realistic model of data; they might also have illustrated the full interpretation of analyses of transformed data. The final chapter on response surfaces is an interesting brief survey, tantalising because too short to allow critical comparison of methods and objectives.

D. J. FINNEY

Plant becomes soil

Biology of Plant Litter Decomposition. Edited by C. H. Dickinson and G. J. F. Pugh. Vol. 1: Pp. xiiv+1-244+46. Vol. 2: Pp. x+245-775+75. (Academic: London and New York, March 1974.) Vol. 1: £7; Vol. 2: £10.50.

RECYCLING of elements bound in living matter is essential to the continuation of life in our, at present, virtually closed global ecosystem. Since plants form the bulk of the world's biomass and, at least on land, the bulk of this biomass is recycled by the decomposition of plant litter rather than by consumption by herbivores, there can be no doubt as to the importance of the subject matter of these volumes. The excellent introductory chapter on 'Litter—Interface of Animate/Inanimate Matter' gives a broad and stimulating view of the whole field of decomposition studies. Some later chapters, by the nature of their narrower

topics, seem by comparison more mundane in their approach to the ecological issues. Following the introduction, the work is in three parts.

In the first part, plant litter decomposition is treated on the basis of different types of plant litter. Thus there is a chapter on lower plant litter, so important in the transition from rock to soil, followed by chapters on decomposition of herbaceous litter, angiosperm tree leaf litter, coniferous leaf litter, wood and roots. Finally, in part I is a chapter on the nature and decomposition of digested plant litter in the form of animal dung and in the faecal pellets from certain soil fauna. It seems artificial to exclude from consideration in the work the decomposition of plant material which has actively been detached from plants by herbivores or by man for feeding to his herbivorous livestock. Such lines of thought, however, lead to the large, but dubiously separable areas of biodeterioration and ruminant nutrition.

In part 2, the chapters survey different groups of organisms involved in plant litter decomposition ranging from bacteria, actinomycetes, terrestrial fungi, aquatic fungi, protozoa, nematodes, oligochaetes, microarthropods, macroarthropods and molluscs, to aquatic crustaceans. The emphasis in these chapters varies: some provide a brief introduction to the classification of the group whereas others assume the reader's familiarity with the group and devote attention directly to the ecological significance of the organisms in litter decomposition.

This approach to ecological problems of plant litter decomposition seems less thought provoking for the general reader than is the approach through litter types or the chapters in part 3 where decomposition of plant litter is discussed in relation to the environment in which decomposition takes place. These last chapters cover decomposition both on the surface of soil and in the depth of soil, in fresh water and in marine environments. The two final chapters discuss special environmental problems of the breakdown of agricultural crop debris and urban waste. Peat and coal are economically important deposits of partly decomposed plant debris. Surprisingly, these are largely ignored in the environmental chapters, though a forthcoming review on peat, by one of the contributors, may account for part of this omission.

The editors have performed a considerable service in bringing together and indicating the relevance of some 3,000 references. I suspect that the work will be appreciated as a source book mainly by students, though researchers in this field will glean grains of information.

R. C. CODNER

Amoral concepts of biology

Philosophy of Biological Science. By David L. Hull. Pp. xi+148. (Prentice-Hall: Englewood Cliffs, N. J. March 1974.) \$6.95.

IN this book, Professor Hull sets out at once to explain to students of philosophy what he regards as the most important laws and concepts of modern biology, and to initiate biologists into a philosophical approach to their problems, therefore assuming in his readers little prior knowledge of either discipline. Hull freely acknowledges the difficulties he has experienced in attempting this in lecture courses addressed to mixed classes of biology and philosophy students, and admits that the aim is a large one for so small a book. It can at least be said that he has achieved his initial aim of making the exposition intelligible to relatively unprepared readers. Students taking courses of the type that Professor Hull delivers will doubtless find this book useful, even though his bibliography is short, highly selective and almost exclusively American.

The extent to which modern professional biologists look to philosophy for guidance in their scientific thought and activity, or to which modern philosophers look to biology as a testing ground for their views on the methods and logic of science, are probably both rather small. One has the impression that biologists tend to develop an interest in philosophy after their main scientific work has finished, generally looking for systems of thought which would justify *post facto* the ways in which they have been thinking. Most philosophers of science, at least within the dominant positivist schools, take the Comtean view, of physics as the paradigmatic science and of biology as a relatively immature and secondary study. It is doubtful whether Professor Hull's book will have any significant influence in changing this situation; neither philosophers nor biologists will find in it any very compelling new insights. Like another recent author in this field, Michael Ruse, Hull seems concerned mainly to provide a responsible articulation of the current American 'conventional wisdom' on the subject. His method of exposition consists in posing a series of philosophical questions about biology, which he proceeds to discuss serially, usually without reaching any very conclusive answers.

Hull's exposition has some very notable omissions—for example, though he is a member of the editorial board of the journal *Systematic Zoology*, and has contributed to it and other journals a series of articles on the theory of biological classification, this topic receives no specific attention in

the book—although most experienced botanists, zoologists and bacteriologists would admit the centrally important role of classification in their sciences (a role with no real parallel in physics), and Michael Ruse devoted two whole chapters to it. Admittedly, one important question posed—but not finally answered—by Hull, does involve the theory of systematics; this question is, to what extent are taxa individuals, with names analogous to proper names, and therefore not permissible in what Hull would recognise as 'laws'? Furthermore, though evolutionary theory is considered at some length, Hull makes no reference to the scientific nature and status of geology—of which it would be quite possible to consider the biological sciences as subordinate parts—nor does he consider the epistemological problems posed by the palaeontologists' claim to knowledge of the organisms of the remote past.

Pride of place in Hull's exposition of biology is accorded to classical and molecular genetics, as domains of what he would accept as scientific 'law'. Yet no reference is made to the profound critique of this conception of genetics by a very eminent geneticist, as expressed in the article "Axiom and Process in Genetics" by C. D. Darlington (*Nature*, **234**, 521–5; 1971). Darlington drew attention, among other things, to the nature of the genotype as the embodiment of race history, and to the dual nature of time, as cyclic and as unidirectionally progressive. Almost the only reference to the nature of time in Hull's book is the offhand remark "Both space and time are now viewed as organisational properties of material bodies"—one of the few ontological assertions he makes. Even the second law of thermodynamics, which might call into question this assertion as far as time is concerned, is mentioned only in dismissing the claim by some authors that organisms can violate it.

Professor Hull's omissions have I think a definite tendency—that of excluding from consideration anything which might raise ontological or moral problems. Recent Anglo-Saxon schools have generally equated philosophy with epistemology, dismissing ontology as "metaphysics" and treating ethics as not properly part of academic philosophy. Hull's failure to consider the moral problems posed by modern biology, and especially by modern medical research, is perhaps a little old-fashioned; there is a rapidly growing general consciousness of these moral issues, not least among students. The issues concern, not merely the ethics of experimentation on human beings, but also the proper relations of man to non-human organisms.

The shortcomings of this book are

shared, to a greater or less extent, by most recent treatments of the subject, and should be attributed less to the author than to the general intellectual climate in which he writes. The essential virtue which Professor Hull can claim is that he at least poses a number of profoundly important questions, the serious pursuit of which by students might lead them far beyond his own exposition—which, after all, is an end that any teacher should be proud to have achieved. R. A. CROWSON

Spoonful of saccharin

Sensory Processes: The New Psychophysics. By Lawrence E. Marks. Pp. x+334. (Academic: New York and London, February 1974.) \$17.50; £8.25.

PEOPLE who use saccharin to sweeten their tea may have noticed a surprising thing: halving the concentration of sugar in a solution reduces its sweetness far more than halving an equally sweet (although much weaker) concentration of saccharin. This is one of many examples that demonstrate differing relationships between sensory magnitude and physical stimulus. How does one measure sweetness, brightness, pitch, odour? It has been shown, particularly in the pioneering work of the late S. S. Stevens, that asking subjects to assign numbers to sensation strength leads to a power function with an exponent dependent upon the stimulus and sensation considered. Lawrence Marks draws a clear distinction between this, the "new" psychophysics, and sensory physics, the "old" psychophysics, in which the observer is simply a detector of threshold, masked threshold, or null point, with measured quantities all in the physical domain. He describes and attempts to interrelate the various psychophysical procedures such as fractionation, category rating, and magnitude estimation and discusses the influences of extraneous factors. The senses are each considered under the headings of sensitivity, temporal and spatial factors, and qualitative aspects. Although one detects a certain antipathy towards the "old" psychophysics it is a carefully reasoned and comprehensive account and shows great concern for validation of the approach. Unfortunately his rather detached attitude coupled with the large number of references makes difficult reading in parts and a certain amount of repetition is inherent in the organisation he has adopted.

Although the new psychophysics gives insights into sensory processes not obtainable in any other way the field has a certain contrived air to it. We do not generally use our senses, or num-

failed, is not that it uses abstract models and traces interdependence between variables, but that it has sometimes been pseudo-planning, confined to a ceremonial superstructure, without being geared to where the action is.

The authors argue for the central importance of the annual budget. Though it is obviously true that no plan can be implemented unless it is integrated into the annual budget, there is a danger of mistaking a necessary, though minor, condition for the strategic one. Budgets are, at best, annual public expenditure plans. A focus on fiscal magnitudes, though essential for proper public accounting, obscures and evades the real activities. Links between fiscal (or even financial) expenditures and results are tenuous, especially in underdeveloped countries. There are no fixed coefficients between money expenditure and land reform, population policy, incomes policy, education, public health, nutrition. Foreign exchange budgeting, manpower budgeting, raw material budgeting are just as important as fiscal budgeting and even they do not exhaust the range of necessary policies. Proper public accounting is necessary to ensure, negatively, that public money is not spent extravagantly or corruptly, but it cannot ensure, positively, that it is spent according to social priorities and that the necessary complementary actions are taken. Budgeting is to planning what bookkeeping is to business management: without it, management is impossible; but with the best book-keeper in the world, a firm can go bankrupt. These points are not enough stressed by the authors, who see the plan essentially as a many-year public capital budget.

Much is made of the need for redundancy. In a piece of exquisite jargon, the authors write: "Broadly speaking, we can regard social poverty as a lack of functional redundancy" (Page 49). But there is a vast difference between reserves (which serve a purpose) and redundancies. A more analytical and quantitative approach would have made the distinction clear. It is now well known that in poor societies, not only unskilled labour, but also capital and technically trained professional manpower are redundant: but, alas, they are not reserves.

The authors make a large number of entirely fair and commonsensical criticisms of planning. "If we were asked to design a mechanism for decisions to maximise every known disability and minimise any possible advantage of poor countries, we could hardly do better than comprehensive, multi-sectoral planning" (Page 293). The need for unavailable information, for political stability, for consistent aims are cited as unattainable condi-

tions. But perhaps the most serious criticism of planning is omitted, viz. that its very success, measured by coherence and consistency, becomes an obstacle to adaptation and innovation. Plans introduce an additional rigidity into societies already inflexible. Plans, for this reason, in spite of their declared intentions, are elements strengthening conservatism.

The conclusion is not, however, reliance on *laissez-faire* and the free play of market forces. The authors rightly point to the need for a combination of contingency planning, continuous budgeting and rolling planning, so that there can be adequate and speedy responses and adaptations to unforeseen events, both favourable and unfavourable.

The authors treat planners and planning as part of the social and political environment which they are supposed to plan. Planning the planners is not an invitation to an infinite regress but a reminder that there must be continuous mutual adaptation between plan objectives and social constraints.

PAUL STREETEN

Details of sense organs

The Ultrastructure of Sensory Organs. Edited by I. Friedmann. (North-Holland: Amsterdam and London; American Elsevier: New York, 1974.) Dfl.90; \$32.70.

THIS collection of four essays is less general than the title implies; only vertebrates are described. Of their sense organs only taste buds (R. G. Murray), the organ of Corti (H. Engström and H. W. Ades), the vertebrate retina (R. F. Dunn) and the olfactory mucosa (P. P. C. Graziadei) are included. The preface indicates the intention of the articles to summarise "the immense and rapid progress that has been made in electron microscopy" for "anatomists, physiologists, pathologists and clinicians". Only Engström and Ades make some attempt to meet the latter two classes of busy people part way. Anatomists are best treated in that all the articles provide descriptive anatomical accounts while remaining substantially innocent of physiological commentary or synthesis of the two approaches.

Thus the essays have to be judged not as summaries of the contributions ultrastructural studies are making to understanding of the mechanisms of action of the organs treated, but as summaries of anatomical information available up until about 1970-71. Most of the summaries are competent but, perhaps because of editorial direction, they all seem of rather even emphasis; so that exciting new developments and the relative importance, or accuracy, of different and differing observations are

hard to discern. Perhaps the main point of the articles was to provide a review of the literature together with an atlas of ultrastructure. There are numerous electron micrographs including a fair number, especially of the retina, that have not previously been published. For the non-specialist in a particular sense organ the essays provide a reasonably readable guide to the literature of the past twenty years. There are useful summary tables of data, for example in the article on the retina. But for the readership suggested, and given that the articles are purely anatomical, a greater use of summary diagrams, perhaps at the expense of some of the electron micrographs, would have been more helpful to ready comprehension. The essays are probably most useful for someone beginning an interest in the structure of a particular sense organ. Most people are likely quickly to prefer the more detailed and often more synthetic articles of the currently appearing *Handbook of Sensory Physiology*.

B. B. BOYCOTT

Computers in mathematics

Computer Approaches to Mathematical Problems. By Jurg Nievergelt, J. Craig Farrar and Edward M. Reingold. Pp. xii+267. (Prentice-Hall, Englewood Cliffs, 1973.) \$8.95.

AFTER an introductory chapter on languages, including some remarks on the parenthesis notation and the polish notation, there is a chapter on combinatorial computing. This includes a discussion of block designs, latin squares, scheduling and tiled rectangles; and also an account of various graph algorithms, for shortest paths, spanning trees and sorting. There follows a chapter on the use of computers in game playing and decision making and another chapter on random numbers and their use in Monte Carlo methods and in simulation. Computing in number theory is next considered and there is a review of the position in the computing of $\sqrt{2}$, e and π . For example, in 1967 the value of π was computed to 500,000 decimal places. Finally there is a section on the use of computers in calculating large prime numbers. The largest known Mersenne prime, by 1971, is $2^p - 1$ where $p = 19937$. The book closes with a chapter on logic and computers.

This book will be of particular interest to the mathematician interested in the use of computers in pure mathematics. But in this connection, it is a little surprising to find no reference to the use of computers in the theory of finite groups. It will also be relevant for students of operational research mathematics.

L. S. GODDARD

Comprehensive mineralogy

Modern Mineralogy. By Keith Frye. Pp. ix+325. (Prentice-Hall: Englewood Cliffs, NJ, 1974.) £6.50.

MANY new mineralogy textbooks have appeared during the past decade; most have been devoted to specialised subjects and these have tended to replace the earlier type, which aimed at providing for the whole of a student's requirements up to the level of the first degree. With advance of knowledge and the need to explain quite sophisticated techniques even in an elementary book, it is no longer possible to provide a comprehensive text at an adequate level without making the volume unduly expensive, cumbersome and forbidding. Specialised books necessarily contain a much fuller treatment than the average student requires at an early stage but the rapid escalation in book prices has now reduced their attraction for purchase by students. To some extent this has restored the need for a new type of comprehensive text and this book is a valiant attempt to meet just this need. It is selective, it omits much of the conventional crystallographic introduction, and its stated purpose is to provide a sound physically-based approach through familiar atomic theory and crystal structures in a logical sequence, and to introduce the student to present-day concepts of mineral formation and equilibrium. How well does it measure up to these rigorous requirements?

There are seven chapters and a 25-page tabular appendix of data on 150 common minerals. Chapter one is a fairly conventional crystal-chemical introduction that considers the nature of chemical bonds, close packing, ionic radii, simple model structures, defects and solid solution. It is pleasant to note the attention paid here to chemical and structural defects, to dislocations and to the evolution of grain boundaries. Chapter 2 provides a systematic account of the crystal structures found in minerals; again it follows a conventional treatment except where the model-structures theme is developed attractively by way of stuffed derivatives to explain many common rock-forming silicates. There follows in chapter 3 an all-embracing exposition of crystal symmetry, including lattices, point groups, the stereographic projection, crystal morphology, twinning and mineral habits, in 60 glorious pages. Logic seems to have quite deserted the author at this stage, for crystal structures have already been explained and many of the topics introduced (such as space groups) are certainly not needed for what follows. Chapters 4 and 5 are shorter; they are concerned with physical properties and the interaction of radiant energy

with crystalline material. Topics dealt with here include mechanical behaviour, radioactivity, surface properties (but not pyro- and piezo-electricity) colour and lustre, atomic absorption, justification of the optical indicatrix, and the principles of X-ray diffraction. Although this is attractively presented, as in chapter 3 it is difficult to understand why so many principles are explained, albeit briefly, when no attempt is made to show how to use them. This middle part of the book is rather like Hamlet without the Prince of Denmark!

Chapter 6 is probably the best part of the entire book; it is excellent. Beginning with a concise and lucid explanation of the phase rule, it passes on to consider phase diagrams. It deals mostly with crystallisation and melting relations in binaries and ternaries, but regrettably does little on 3-phase triangles or on working out detailed courses of crystallisation. It then introduces isothermal and isobaric sections (used in the final chapter) and then passes on to a brief consideration of a quaternary system with a vapour phase. The final chapter spans an even vaster canvas—the whole of petrology! It begins with an enunciation of the principles of geochemistry and mineral formation and then proceeds to consider the classification and petrogenesis of igneous, sedimentary, and metamorphic rocks as well as some aspects of ore deposits. Although much of the treatment here is skilfully conceived as a consummation of all that has gone before, the familiar symptoms of extreme compression and a plethora of quite difficult new concepts, criticised earlier, here becomes a veritable disease. This must render the chapter virtually useless to an elementary student as a first reading. The repeated misuse of 'lattice' for 'structure' here is deprecated. I cannot see any value in the appendix, which is not sufficiently comprehensive to be of much use for identification yet takes up valuable space.

The diagrams are clear and well drawn but a few minor errors were noted (Fig. 6.13a). Separate author, mineral name and subject indexes are provided.

The author deserves full credit for producing an original, thoughtful and always interesting book which omits much of the irrelevant and otiose morphology still on offer in many. I do not wish to be pejorative but far too much has been attempted, the result being a book that is not really adequate as a crystallography, mineralogy or petrology text. It does not explain how to determine a point group, analyse a stereogram, use a polarising microscope or obtain information from an X-ray photograph. It does not explain the detailed reading of phase diagrams or

how to describe a rock. Nevertheless it contains much information of value not easily found elsewhere and sections can be strongly recommended as further reading by more mature students and as a source of good ideas for elementary teaching. The author has demonstrated his ability to explain difficult ideas clearly, but the book would have fulfilled its rôle as a mineralogy text better if the final chapter and appendix had been omitted, the space saved being used to explain practical aspects of optics including some mention of reflected light, to expand the X-ray section, and to say more on solid solution in rock-forming minerals.

I. D. MUIR

Microscopy for all

The Encyclopedia of Microscopy and Microtechnique. Edited by Peter Gray. Pp. xi+638. (Van Nostrand Reinhold: New York and London, January 1974.) £16.25.

IN recent years methods of microscopy and microtechnique have expanded at such a rapid rate that it is almost impossible to remain abreast of current developments in all but a small area of specialisation. This not only makes the provision of extensive reference books essential, it renders the task of their compilers almost impossible. The present book is, as the editor states in the preface, an attempt to cater for the needs of all who use the microscope, not only in the medical and biological sciences, but also among the "atmospherologists, bakers, chemists, metallurgists, textile workers, the manufacturers of paints and those who examine paintings". With such an implied wide-ranging coverage in mind, the need for interdisciplinary communication (especially with regard to such a basic tool as microscopy) is clearly apparent and the attempt is praiseworthy. In practical terms, however, it seems that it is not entirely successful. One wonders what the textile worker or paint manufacturer who uses the microscope will gain from the inclusion of a long description of the morphology and systematics of the annelids.

An attempt to cover the whole spectrum of possible microscope usage must fall far short in some fields and it seems that the basic fault of this book lies here. Had the coverage been limited to the instrument and to basic preparative techniques, the value of the book to all users of the microscope would have been increased and the topics could have been covered in more detail.

Most of the articles in this book (provided by about 180 specialists) are short, only one or two pages in length,

and so cannot provide more than a very superficial coverage of the subject. There is, however, a list of references after most articles which serves as an introduction to sources which provide more detailed information. As might be expected, there is a considerable variation in the standard of the individual articles. Some provide an excellent thumb-nail sketch of the subject whereas others contrive to say very little indeed. The production quality is good and there are surprisingly few misprints for such a large book (although some inconsistencies have crept in, Abbe's name being rendered variously as Abbé or Abbee for example). The index is an essential feature of any reference book and in this case it is perfectly adequate.

S. BRADBURY

Raman analysis

Applications of Laser Raman Spectroscopy. By Stanley K. Freeman. Pp. xi+336. (Wiley: New York and London, January 1974.) £9.75.

THIS new book on the applications of Raman spectroscopy is the most significant modern volume to survey the analytical value of laser Raman spectroscopy. It includes a compendium chapter on the principles behind the Raman and related effects and others on experimental technique, a long section on qualitative analytical applications and concludes with chapters on synthetic and biopolymers and the value of Raman methods in pollution studies.

Molecular vibrations are normally studied by infrared absorption and Raman scattering. Since the vibrations characteristic of a complex molecule are themselves complex, these two techniques can, in principle, provide excellent fingerprints of analytical value. Infrared spectroscopy has been used routinely in this way for more than thirty years but Raman spectroscopy has not. Forty years ago Raman spectroscopy was thought to have immense analytical potential but it became clear in the later 1930s that experimental limitations severely restricted the versatility of the method. The relatively recent advent of the laser and sophisticated commercial spectrometers have improved the situation dramatically with the result that Raman spectroscopy is now playing an invaluable part both as an analytical tool (in the broadest possible sense), in structural chemistry, solid state physics and many other diverse areas.

In his book, Freeman selects some of the analytical applications of Raman spectroscopy and discusses these in considerable detail. Since other books exist covering infrared and Raman

spectra of crystals, and reviews abound in other detailed areas, he quite rightly spends most of his time on the area in which he is best known and which is worst surveyed elsewhere: the use of Raman spectroscopy for functional group identification in organic analysis. In this area, the book is unique and contains a wealth of otherwise unpublished data and the significant results of years of experience. In fact, it can be truthfully said to be the logical successor to the classic books in this field by Hibben and Kohlrausch, both published before 1940!

In the chapters on polymeric and biological compounds the coverage is again chemical and analytical rather than structural and little space is spent in considering the niceties of vibrational theory and structural consequences thereon. But in the context of the book as a whole, this is entirely relevant. The section on pollution monitoring and Remote Raman techniques is also valuable in that it surveys a field which is very diversely covered in the literature.

My initial reaction to Freeman's book was most unfavourable since the title is misleading. Nowhere convenient does the author point out that to him "applications" mean analytical ones, thus many will be disappointed by the narrow coverage and extravagant claims on the dust cover. Further, the production quality is reminiscent of a cheap paperback. But once the first chapter is digested and the general tenor of the coverage grasped, the book endears itself to the practicing chemical vibrational spectroscopist.

P. J. HENDRA

Aluminium analysed

Analytical Chemistry of Aluminum. By V. N. Tikhonov. Translated by J. Schmorak. Pp. x+303. (Analytical Chemistry of the Elements.) (Halsted, Wiley: New York, Toronto and Chichester; Israel Program for Scientific Translations: Jerusalem, January 1974.) £9.55.

THIS latest volume in the series of monographs dealing with the analytical chemistry of the elements, and sponsored by the USSR Academy of Sciences through the Vernadskii Institute of Geochemistry and Analytical Chemistry, is concerned with aluminium (*sic*).

The contents follow the general pattern established in previous volumes. A short preliminary chapter details relevant information about the occurrence, the physical and chemical properties of aluminium and its compounds, particularly the properties of complexes of the element with organic compounds which have found use as

analytical reagents. Chapter 2 contains an extensive account of chemical and physicochemical methods for the determination of aluminium, in which gravimetric, titrimetric, photometric and fluorimetric methods are given comprehensive coverage, rather less space being devoted to polarography, radioactivation and spectroscopic methods. Chapter 3 deals with the separation of aluminium from accompanying elements by precipitation with inorganic and organic reagents, by extraction, by a variety of chromatographic methods and by mercury cathode electrolysis. Chapter 4 treats the determination of aluminium in natural and industrial materials such as minerals, ores and industrial concentrates, soil, water and organic substances, metals and alloys. Chapter 5 brings the text to a conclusion with a description of methods for the determination of impurities in high-purity aluminium. The bibliography of work cited in the text contains over 1,300 entries. As with the earlier volumes in this series, the translation has been expertly done.

As the third most abundant element in the Earth's crust, aluminium is present in most natural and fabricated materials. Although its chemistry and hence its analytical chemistry is not particularly complicated, a vast literature on methods for its determination, particularly in the presence of interfering elements, now exists. Surprisingly, no attempt has been made to review this information since 1942, when Fischer contributed a monograph on the subject in the *Handbook of Analytical Chemistry* by Fresenius and Jander. The present text deals mainly with the literature up to 1968 with occasional later references, none, of course, beyond 1971, the year of publication of the original Russian version, and covers all known methods for the determination of aluminium.

There can be no doubt about the value of this type of monograph to the practising analyst. Many of the problems associated with the analysis of aluminium—the ignition temperature of aluminium oxide, the pH for precipitation of aluminium 8-hydroxyuinate, the difficulties of the complexometric titration, the volatilisation of aluminium for AAS, the best photometric reagent for a particular purpose—all are given thorough and realistic treatment. Although this book is essentially a review of existing literature, perhaps a major benefit is its presentation of Russian work not readily accessible to analytical chemists in the Western World. As a comprehensive and authoritative source of information on the analytical chemistry of aluminium, it must assume the premier role.

W. I. STEPHEN

Science in the media

Two contrasting looks at the place of science reporting in the media—one from each side of the Atlantic—have recently combined to provide an insight into the delights and pitfalls of science journalism, while showing how different these are in different countries. In Britain, the BBC Radio 3 programme "The Communicators" looked at science reporting in its issue of July 27. Here, the emphasis was squarely on the difficulties involved in getting a science story into the British national press at all.

The situation in this respect has deteriorated over the years, as the long serving science correspondent of the Daily Express recalled. With shortage of newsprint and a feeling that the public is to some extent disenchanted with hard science, it is all too easy for science journalists to fall into the trap of overdramatisation, at least in the eyes of the scientists. Several scientists interviewed in the programme expressed their concern about sensational reporting—one was distressed by a comment from one of the people making a documentary about typhoid that "we are in this game to entertain the public, not to educate them".

Such concern about sensational and inaccurate reporting is also felt on the other side of the Atlantic, to judge from an 'occasional paper' on *Science in the Newspaper* which has recently been published by the American Association for the Advancement of Science. But in many other respects the situation in the USA seems far different from that in Britain, giving the lie, perhaps, to any suggestion that sensational reporting arises because of the difficulty of getting any science story into print.

Science in the Newspaper itself is an odd mixture; contributions from award winning writers discussing their craft rub cheek by jowl with 'scientific' investigations of science reporting, complete with jargon and footnotes. Whether by accident or design, this neatly emphasises one point: the scientists certainly do need help in communicating their ideas to anyone outside a restricted circle of specialists.

Perhaps the most worrying contribution describes the result of a survey of what scientists think about science in the newspaper. The scientists had been asked to check for errors published in stories about their own work, and in more than 25 per cent of the cases they noted such damaging errors as misquotations, misleading headlines, omission of relevant information and excessive brevity.

But are the criteria scientists apply too strict, bearing in mind the audi-

ence for which the articles were intended? When one respondent objected that in a report of the drop out rate of college students the first sentence said that more than half of the college students 'failed to obtain degrees' when it should have been qualified to add 'within four years', the criticism seems valid; but, we are told, the report in question did actually contain the important qualifications in its next sentence. That was not good enough for the scientist whose work was being described, but I would find it difficult to see his objection as one to worry the reporter who made the 'error'.

The point is, of course, that newspaper readers, by and large simply speak a different language from scientists, and that the journalist is literally in the position of interpreter. So many of the irritations felt by scientists are on the same level as the quibbling among multinational organisations about exactly how a French word in an agreement, say, should be translated into German.

This interpretative role is made abundantly clear in the most heartening article in *Science in the Newspaper*. The *National Enquirer* is an American weekly that was until a few years ago, we are told, a 'scandal sheet'. But now the *Enquirer* has changed its image, become a 'family newspaper' and just about quadrupled its circulation, to 4 million. And in that large circulation paper, science has a vital role.

In one recent 48 page issue mentioned, five per cent of the space was given up to science or medical stories. The treatment of these stories is dramatic, and successful in reaching a wide audience who respond with floods of letters. Not all the scientists whose work makes the pages of the *Enquirer* are pleased—Dr Sol Spiegelman, of Columbia University's Institute of Cancer Research, is quoted in *Science in the Newspaper* as being particularly unhappy about the treatment he received from the *Enquirer's* reporters.

But you can't please all the people all the time, and if a paper like the *Enquirer* can, by and large, encourage its readers to accept science as a valuable part of modern society which deals with problems that are relevant to the man in the street, the science journalists are doing their job. The success of the *Enquirer's* science policy ought to make some British popular newspapers rethink their attitude to science; the scientists might not always approve of the result, but then, non-scientists do not always approve of the activities of scientists.

The message from both of these slightly narcissistic looks in the mirror

emphasises a feeling which already exists in both the USA and the UK. Science today must be relevant to the problems of today, and what is more it must be seen to be relevant.

JOHN GRIBBIN

Easy listening science

Over the past few weeks Saturday morning listeners to BBC Radio 4 have been having a taste of up to the minute reports from the scientific world. "Science Now", as its title suggests, has been keeping its audience abreast of the latest developments in science and techniques involved in anything from building a skyscraper from the top downwards to the possible hazard to the public from the development of new strains of mutant cells.

The BBC's radiophonic workshop provides a snappy piece of introductory music which sets the tone for a tightly packed programme of comment, interview and discussion. The presenter, Brian J. Ford, manages to maintain the tempo throughout, and it is this which seems to make the programme such easy listening.

Each thirty minute programme can cover a range of scientific topics acceptable both to working scientists and to the man in the street with an interest in science. When 'test tube babies' hit the headlines recently, "Science Now" covered the subject and brought out the implications in non-sensational terms. As Brian Ford said, the work represents "an interesting development, but not by any means a terrifying prospect".

In the same programme the question of how massive the Universe is and the eventual slowing down of its expansion, was discussed. But as seems inevitable in any science programme today, medical items receive a good share of attention. Audience response indicates the understandable popularity of medicine, but it does seem a little unfortunate that this sometimes serves to divert a large proportion of the very limited air time available for science (see *Nature*, 250, 362; 1974) away from 'hard science'.

Still, a complete edition of "Science Now" was devoted to applied science and included oceanography, crop irrigation, building methods, cancer research and an exhibition to celebrate the discovery of oxygen 200 years ago.

Where the programme differs from some of its predecessors is in the amount of comment provided by the presenter, either with or without contributions from a second party. This is a significant and worthwhile development, since often the caution of a

scientific paper, which might be the peg for an item, masks an exciting development. Equally, of course, Ford has scope to comment about developments, such as the 'test tube baby' story, where exciting publicity has masked the reality of a small and cautious scientific step, forward.

Given that such a programme must, to a large extent, reflect the personal approach of presenter and producer, "Science Now" is doing a good job. It seems unfortunate, however, that it is broadcast only once a week, at a time when many would be listeners must be involved with the ritual of

weekend shopping and/or sport. Although much of the same ground is covered by *New Scientist* or the national press, it would still be good to have a second opportunity to hear the programme, perhaps on a weekday evening.

SALLY OWEN

obituary

Chu K'o-Chen

CHU K'O-CHEN, one of China's most distinguished scientists, Vice President of Academia Sinica, died last February at the age of 84. When I first met him in 1944 he was President of Chekiang University, then in exile in the little town of Mei-t'an in Kweichow Province. One could soon glimpse the qualities which endeared him to generation after generation of Chinese scientists—a real lover of the things of the intellect and of Nature—small of stature, kindly, judicious and compassionate.

His original training in geography, meteorology and astronomy was gained at the University of Illinois in 1917 and subsequently at Harvard. After he returned to China he became Director of the National Meteorological Institute of Academia Sinica at Nanking. Later on, after the Second World War, he was called from Chekiang University to become one of the Vice Presidents of the newly reconstituted National Academy of Sciences. Apart from his early publications in geography and the

techniques of meteorology, he acquired a life-long interest in phenology (historical meteorology). Thus it came about that Chu K'o-Chen led a whole group of scientists in the study and analysis of China's climatic changes over more than 5,000 years.

Another great interest of Chu K'o-Chen was the history of astronomy in Chinese culture. He published a number of papers on such subjects as the origin of the twenty-eight lunar mansions (*hsiu*) and the development of calendrical science. It was natural, therefore, that he should exercise a beneficial influence on such studies in China, and he was one of the founders of the Institute of the History of Science some years ago in Peking, where it occupies the lovely old buildings of a former Manchu prince's palace.

Many Western scientists who have worked in China have been greatly indebted to Chu K'o-Chen for his kindly understanding and never failing help. Two pictorial reminiscences of him come especially to my mind. First at Ts'un-yi, giving a warm welcome to

some wandering European scientists in 1944 with the aid of the delicious *pao-tzu* (stuffed dumplings) made in that place and cooked traditionally on beds of pine needles. Secondly, at the International Congress of the History of Science at Florence in 1956, Chu K'o-Chen led a distinguished Chinese delegation. When we all went out to Vinci to pay our respects at the farm where Leonardo was born, and to visit the castle of the little town with its museum of Leonardo's inventions, we, as an international convention, received an unexpectedly enthusiastic welcome from the *podestà* and the people of Vinci. They regaled us with a splendid lunch and with an *al fresco* supper where they pressed upon us flasks of the excellent local wine. The Chinese and Japanese delegates, having come from so far away to pay homage to Leonardo, were particularly appreciated by the *cittadini*, and the sight of Chu K'o-Chen and his colleagues leaving for the bus with as many flasks as they could possibly carry remains one of my happiest recollections in the world of international science and its history.

Announcements

Awards

Alasdair Muir Breckenridge has been awarded the **Paul Martini Award 1974** by the Medizinisch Pharmazeutische Studiengesellschaft eV, Frankfurt.

Chaim Leib Pekeris has been awarded the **1974 Vetlesen Prize in Earth Sciences** by the University of Columbia.

Appointments

Bernard Salvage has been appointed to a personal chair of high voltage engineering at **Heriot-Watt University**.

W. A. Cramond has been appointed Principal and Vice-Chancellor of the **University of Stirling**.

Errata

In the article "Elongation factors for chloroplast and mitochondrial protein synthesis in *Chlorella vulgaris*" by O. Ciferri and O. Tiboni (*Nature new Biol.*, **245**, 209; 1973) some of the references in the text were wrongly numbered. The following corrections should be made: in Table 1 and Fig. 1, 3 should be 6; 4, 7; 5, 8; 6, 9; 7, 10; in Fig. 2, 9 should be 12; in the text on pages 210 and 211, 10 should be 13; 11, 14; 12, 15; 13, 16; 14, 17; 15, 18; 16, 19; 17, 20; 18, 21; and 19, 22.

In the article "Dietary preference and diseases of age" by M. H. Ross and G. Bras (*Nature*, **250**, 263; 1974) an error appeared in Table 1. The third number down in the penultimate column should read 77" not 7".

Corrigenda

In the article "Early visual adaptation in goldfish retinal ganglion cells" by A. J. Afanador and A. J. Adams (*Nature*, **250**, 346; 1974) the last line of the legend to Fig. 2 should read "0 corresponds to $7.2 \times 10^{15} \text{ s}^{-1} \text{ cm}^{-2}$ ".

The following corrections should be made to the article "Platelet contractile regulation in an isometric system" by I. Cohen and A. de Vries (*Nature*, **246**, 36; 1973); line 41, $0.2 \text{ g min}^{-1} \text{ cm}^{-2}$; line 43, 1.8 g cm^{-2} ; Fig. 1 "... expressed in g cm^{-2} "; Fig. 2 ordinate Tension (g cm^{-2}) legend, "... expressed in g cm^{-2} "; Fig. 2 ordinate Tension (g cm^{-2}); Fig. 4 legend, Clot contraction model.